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ACCIDENTS FOLLOWING THE SUBDURAL INJECTION OF THE ANTIMENINGITIS SERUM.

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The antimeningitis serum has been employed with constantly increasing frequency since 1906-1907, and those physicians who have come to have the widest experience in its use are unanimous in the opinion of its high therapeutic value. Elaborate reports¹ have now been published from America, France, Germany, and still other countries, and they agree in indicating that the reduction in mortality caused by epidemic meningitis and brought about by the serum is from two-thirds to three-fourths of the average percentages occurring in the same periods and places among the patients not subjected to the specific treatment. There is, at present, no other effective means of treatment of the disease known. It becomes, therefore, a matter of great importance to discover and then to ascertain the reasons for any harmful effects arising in the course of the serum treatment and attributable to the treatment itself.

That an immune horse-serum can be safely injected into the cerebrospinal meninges in the lumbar region was first shown in connection with the antitetanic serum, which has been repeatedly injected in this manner without causing, so far as the reported cases indicate, untoward symptoms. That the antimeningitis serum can be administered with safety in the same manner is attested by large numbers of instances among children and adults who have been treated with the serum for suspected or established epidemic meningitis. But that accidents may sometimes attend the subdural injection of a foreign serum is obvious. The meninges are in such close anatomic relationship with the central nervous organs that

1. For bibliography see Flexner: The Results of the Serum Treatment in 1300 Cases of Epidemic Meningitis, Jour. Exper. Med., 1913, xvii, 553.

what affects the one may come to affect the functions of the other. Moreover, in rare instances the injection of horse-serum itself has, as is well known, exerted an injurious action. It becomes necessary, therefore, not only to ascertain what the accidents are that may follow the administration of the antimeningitis serum, but to determine their causes and thus to separate those which are avoidable from those which, at present, may be unavoidable.

We may dismiss as unimportant the ordinary forms and grades of serum disease that follow the subdural as well as the subcutaneous or intravenous injection of horse-serum; but the mere fact of their occurrence should make us alert to the possibility that the severer effects of so-called anaphylactic shock may also, even if rarely, arise. Such an effect was indeed first described by me;² but it will perhaps be most useful to consider the severer types of accidents thus far reported. They have been, on the whole, small in number and may be considered according to the several explanations that have been offered for them. They have been variously attributed, first, to anaphylaxis; second, to rapid lysis of the meningococci; third, to excessive intracranial pressure; and fourth, to poisoning by the phenol preservative sometimes present in the serum. Certain physicians having had large experience with the serum have not reported them at all. In one or two instances, on the other hand, the number reported has, as compared to the number of cases treated with the serum, been large. This disparity itself emphasizes the necessity for a careful and critical study of the circumstances under which the severe symptoms have arisen.

I prefer to begin this consideration with the fourth possibility, since it has been brought forward by Dr. S. P. Kramer to explain several unfortunate results which came to his attention in connection with the administration of the antimeningitis serum in the Cincinnati Hospital.³ Dr. Kramer has undertaken to account for them on the grounds that the serum in certain individuals is delivered into an open central canal of the spinal cord, through which it reaches in

2. Flexner and Jobling: Serum Treatment of Epidemic Cerebrospinal Meningitis, *Jour. Exper. Med.*, 1908, x, 141.

3. Kramer, S. P.: A Possible Source of Danger in the Use of Antimeningitis Serum in Young Children, *THE JOURNAL A. M. A.*, May 3, 1913, p. 1348; *Lancet-Clinic*, 1913, cix, 304.

a concentrated form the fourth ventricle, at which point the phenol preservative has ready access to the respiratory center in the medulla, on which it acts injuriously, with the result that respiration is paralyzed. This explanation assumes, first, that the central canal of the spinal cord is open in many individuals and, second, that the disturbances of respiration are poisonous or toxic in origin. In order to support his hypothesis Dr. Kramer performed several experiments on dogs. The experiments consisted in the injection into the vertebral artery of a phenolized serum, or in surrounding the exposed medulla with a solution of phenol in water or serum, which procedures are said to have given rise to symptoms of respiratory failure simulating those following the subdural injection of the antimeningitis serum. Since no actual relationship exists between the conditions of these experiments and the conditions occurring in the subdural injection of the antimeningitis serum in human beings, they need not be regarded seriously; and this is particularly true since, as will immediately be shown, it is superfluous to invoke the presence of the phenol in the serum in order to account for the serious results mentioned.

Thus, if the explanation offered by Dr. Kramer is the correct one, no accidents should arise when a serum containing no preservative whatever is employed for subdural injection. The antimeningitis serum produced in France is prepared at the Pasteur Institute by Dr. Dopter and is called by his name. It is supplied in sterile containers, without preservative, and is merely stored for a time before use in order to remove the complement and reduce the risks of ordinary serum disease; and yet, in rare instances, serious symptoms, recalling those described by Dr. Kramer, have followed the administration of Dopter's serum. While it is true that the accidents are not, as a rule, fatal, yet in a very few cases death followed within a short time after the injection. It is, moreover, significant that the injection of the phenolized serum into the lateral ventricle is borne quite as well as its usual injection into the lumbar meninges. Now there does not exist a more direct path to the fourth ventricle than that of the cerebral ventricles. If, therefore, the mere presence of the phenolized serum within the fourth ventricle in children sufficed to bring about severe poisonous effects we should look for

such a result from the intraventricular injections. And yet, not only has this not happened, but the latter injections have proved in several remarkable instances to be life-saving.¹ The accidents, moreover, have not been limited to children but have been observed in adults in whom a patent central canal of the spinal cord, if it exists at all, must be an extremely rare occurrence.

The experience with Dopter's serum, as well as with the intraventricular injection of the phenolized serum, disposes once and for all of the hypothesis of a toxic effect as accountable for the symptoms. We turn, therefore, to the consideration of anaphylactic shock resulting from the administration of the antimeningitis serum. In an early publication we described such an instance in a young child who, after receiving several injections of the serum in close succession without disturbing consequences, reacted severely to an injection made after an interval of sixteen days to counteract a relapse of the infection. The result was an acute anaphylactic reaction from which the child recovered only to succumb some days later to the meningitis itself. Since then some other instances of supposed anaphylaxis have been described in patients who had previously been injected with diphtheria antitoxin, or in whom, because of relapse, the injections of the antimeningitis serum have had to be resumed after intervals of days or weeks.⁴ What is particularly impressive and reassuring is the fact that these severe reactions, which theoretically, have been very much feared, have been, on the whole, very infrequent and, generally speaking, not fatal.

But severe symptoms have also been noted to follow a first injection of the serum. The question therefore arises as to whether these have been in all instances examples of anaphylaxis corresponding to the severe shock that occasionally follows a first subcutaneous injection of diphtheria antitoxin.⁵ This is indeed the crucial point.

4. The reported fatal cases of this class have been as follows: Levy, *Klin. Jahrb.*, 1911, xxv, 188, one; Hutinel, *Presse méd.*, 1910, xviii, 497, two, one of which was complicated by tuberculous meningitis; Debove (cited by Hutinel, *loc. cit.*, p. 498), one; Chevrel and Bourdinière (*L'hyg. gén. et appliq.*, 1910, v, 20), one.

5. This has been the prevailing view. But the especial susceptibility of patients in a desperate condition at the time of injection, that is, fulminating or very severely ill and usually unconscious subjects, should arouse suspicion.

In certain of the instances reported by Dr. Kramer and Dr. Parmelee⁶ a severe reaction followed, not on the first, but on the second or third injection of the serum, given at brief intervals. In these instances anaphylactic shock can be excluded. Dr. Parmelee kindly supplied me with a brief account of the cases from which I derive this conclusion. If, then, the effects under discussion are not due either to the poisonous action of phenol or to anaphylaxis, what is their cause?

In the course of the immunization of horses with cultures of the meningococcus for the purpose of producing the antimeningitis serum the fact has been noted that the intravenous injection of the cultures, which at first is well borne, later becomes a source of danger to the animals. That is to say, after a certain degree of immunity has been established the further injection of the meningococcus cultures directly into the veins produces such a severe effect on the animals that some of them die suddenly within a short time after the inoculation. In order to avoid this result it has been found necessary to carry on the immunization of horses by the slower but safer subcutaneous injection of the culture. Dopter has been especially impressed with this phenomenon and has undertaken to explain it on the supposition that the immune animal possesses the power of rapidly dissolving the introduced meningococci, through which lysis there is set free such a large amount of dissolved toxin that the animal dies of an acute poisoning. In support of this view he has observed that when guinea-pigs are inoculated with a mixture of meningococci and immune serum they succumb in a short time and in a manner recalling the sudden death of the horses; while this effect is not observed⁷ when a corresponding amount of the menin-

Netter (*Compt. rend. Soc. de biol.*, 1910, lxix, 12) has reported four such fatal cases among 100 patients treated by him; Hohn (*Klin. Jahrb.*, 1908, xx, 368), one case among thirty-seven; Martin, of the Pasteur Institute Hospital (*Paris Letter, THE JOURNAL A. M. A.*, Nov. 16, 1912, p. 1807), two cases among twenty treated, with which are to be compared the 7 cases of Dr. Kramer and the 8 cases of Dr. Parmelee. Most observers have not experienced them. Jehle (*Wien. klin. Wchnschr.*, 1909, xxii, 619) refers them to status lymphaticus.

6. Parmelee, A. H.: Epidemic Cerebrospinal Meningitis, *THE JOURNAL A. M. A.*, March 1, 1913, p. 659.

7. Briot and Dopter: Pathogénie des accidents observés au cours de l'immunization des chevaux contre le méningocoque, *Compt. rend. Soc. de biol.*, 1910,

gococcus culture is inoculated without the serum. On the basis of these observations an attempt has been made to explain the severe symptoms sometimes following the first injection of the antimeningitis serum on the supposition that the serum brings about such a rapid dissolution of the meningococci within the cerebrospinal exudate as to cause an acute poisoning of the nervous system. This explanation is surely inadequate to account for most instances observed in man, since it does not apply to the cases in which severe symptoms arose but in which the number of meningococci within the inflammatory exudate has been very small and altogether insufficient to give rise to the soluble toxin in question, or in which the meningitis has been caused not by the meningococcus but by the pneumococcus.⁸

There remains one other possible source of the accidents, namely, that of increased intracranial pressure. In this connection it should be recalled that the severest symptoms relate to disturbance of respiration and in a far slighter degree to the heart's action. Usually the respiration fails while the heart may continue still to act well. For this reason artificial respiration has frequently sufficed to restore the patient. That a dangerous degree of increased intracranial pressure might be produced by the subdural injection of the antimeningitis serum was recognized at the inception of the serum treatment, whence it was advised to inject less serum than cerebrospinal fluid removed and not to employ maximal doses.² Because of the rarity of serious effects this warning has fallen more and more into disuse until now the rule is to give far larger doses of serum than were originally employed and often to disregard the relation of dose of the serum to age of the patient. It is necessary always to keep in mind the dangers arising from increased intracranial tension. Cushing, who has especially studied the condition experimentally and in human patients, states that the medullary

lxix, 10; Action expérimentale du sérum antiméningococcique sur le méningocoque, *ibid.*, p. 126. Dopfer, Ch.: Action bactériolytique comparée du sérum antiméningococcique sur les méningocoques et les germes similaires, injecté par voie veineuse, *ibid.*, p. 524; Le pouvoir lytique du sérum antiméningococcique est-il spécifique?, *ibid.*, 546; Différentiation du méningocoque et des germes similaires par l'“épreuve du péritoine,” *ibid.*, p. 600. -

8. Netter, A.: Compt. rend. Soc. de biol., 1910, lxix, 12, 166. Netter, A., and Debré, R.: La méningite cérébro-spinale, Paris, 1911, p. 261.

center of respiration already impaired from compression endures an additional burden less well than a medulla not so embarrassed. He observed that in death from a fatal increase of intracranial tension the arrest of respiration precedes that of the heart; and he pointed out that in clinical states representing conditions of general compression, among which is acute meningitis, the tension of the fluid in the cerebral meninges more nearly corresponds with the degree of pressure capable of producing the symptoms of compression.⁹

We see, therefore, a close relationship between the effects of increased intracranial tension and the serious symptoms which have sometimes arisen on the injection of the antimeningitis serum. Koplik¹⁰ early suspected this danger and therefore advised the substitution of the injection of the serum by gravity for injection by syringe. The employment of the gravity method has another important advantage, namely, that it permits the immediate withdrawal of fluid at the first sign of any embarrassment to the function of respiration. Recently a further safeguard has been introduced by Sophian,¹¹ who employs the registration of the blood-pressure to supply an ocular guide to the injection of the serum by the gravity method. Sophian has probably had the largest single experience in the personal administration of the antimeningitis serum and he reports some 1,500 injections without a single serious accident. From the effort to guard against this preventable danger Dr. Kramer has made the following wholly gratuitous assumption in his paper: "Indeed, so common has this catastrophe become that in a circular issued by the New York Department of Health, directions are given as to what should be done when breathing stops following the administration of the serum." The Commissioner of Health of the New York Department of Health has already replied to this unwarranted statement and intimated that the instructions for the

9. Cushing, H.: Mütter Lecture, 1901, Some Experimental and Clinical Observations Concerning States of Increased Intracranial Tension, *Am. Jour. Med. Sc.*, 1902, cxxiv, 375.

10. Koplik, H.: The Serum Treatment and the Prognosis, under Various Forms of Therapy, of Cerebrospinal Fever, *Med. Rec.*, 1908, lxxiv, 557.

11. Sophian, A.: A New Method for Controlling the Administration of Serum in Epidemic Meningitis, *THE JOURNAL A. M. A.*, March 23, 1912, p. 843; *Epidemic Cerebrospinal Meningitis*, St. Louis, 1913.

administration of the serum were prepared to avoid the dangers the disregard of which may be followed by such serious consequences.¹²

If we now return to the consideration of the circumstances surrounding the treatment of the cases reported by Dr. Kramer, as well as those referred to by Dr. Parmelee, we at once notice the heaping up of unhappy accidents, while similar ones have arisen with rarity in other places. This fact alone suggests causes local in nature, since it can hardly be imagined that the extraordinary experience is the result either of anatomic conditions in the patients or peculiarities in the infecting meningococcus. So far as I have been able to make out, the doses of serum employed in the cases reported by Dr. Kramer, as well as those referred to by Dr. Parmelee, have, almost without exception, been maximal, and this is particularly true among the children in whom severe symptoms developed.¹³ Moreover, there is no indication of the use of safeguards in the administration of the serum. The injections, as far as is stated, were made with a syringe. In a number of instances the accident occurred, not at the first, but at some subsequent injection of the serum, which

12. Lederle, E. J.: *The Treatment of Cerebrospinal Meningitis with Specific Serum*, Lancet-Clinic, 1913, cix, 485.

13. Dr. Kramer's patients received serum as follows: In a child of 10 months 20 c.c. of cerebrospinal fluid were removed, and 20 c.c. of serum were injected; in one of 4 years 30 c.c. of cerebrospinal fluid were removed and 30 c.c. of serum injected; in one of 3½ years 20 c.c. of fluid were removed and 15 c.c. of serum injected; in one of 7 years, 45 c.c. of cerebrospinal fluid were removed and 40 c.c. of serum injected; in one of 5 years, 25 c.c. of cerebrospinal fluid were removed and 37 c.c. of serum injected; a second injection of 30 c.c., on the next day, produced no symptoms; the following day 30 c.c. of fluid were withdrawn and 30 c.c. of serum injected, followed by death; in one of 10 years, 40 c.c. of cerebrospinal fluid were removed and 40 c.c. of serum injected; in one of 9 years, 50 c.c. of cerebrospinal fluid were removed and 40 c.c. of serum injected.

Dr. Parmelee's patients who succumbed after the first injection were adults. There is no record of the quantity of cerebrospinal fluid removed. They received respectively 45 c.c., 45 c.c., 40 c.c., and in one case the quantity is not mentioned. The patients who succumbed after the second injection included a child of 5 years, in whom the first dose was 20 c.c. and the second 15 c.c.; an adult of 50, in whose case the record states merely that 45 c.c. were given in all and does not refer to the cerebrospinal fluid removed; an adult of 40 years, who is said to have received 30 c.c. in all; and a child of 2 years who received 25 c.c., and twelve hours later 20 c.c., and regarding whom it is not stated whether or not cerebrospinal fluid was removed.

itself is hardly consistent with the supposition of the action of a toxic agent. Altogether, it seems to me that the unhappy experiences can be explained more satisfactorily on the supposition that they resulted from increased intracranial pressure than that they were examples of anaphylactic shock. Since, in some instances, the severe symptoms did not arise on the first injection, but on the second or third injection of the serum, given in close succession, it is quite certain that anaphylaxis played no part. It is equally certain, in view of what has already been stated, that the phenol preservative can be excluded. There remain, therefore, two possibilities; namely, first, that the severe effects were caused by toxins liberated by the immune serum from the meningococci, which then must have been present in large numbers, or, next, that they were the result of excessive intracranial pressure. That they were not caused by meningococcus toxin is shown by the fact that in some of the reported cases the cerebrospinal fluid removed contained either few microorganisms or even none that were demonstrable in microscopic preparations. Hence, the indications are that either in all the instances, or in practically all, excessive intracranial pressure was the source of the accidents.

We must not lose sight of the essential fact that in epidemic meningitis the struggle is with a highly fatal disease and the one means now possessed to combat it is the antimeningitis serum. From this it follows that it is not justifiable to withhold the remedy in spite of some small risk, in view of the far greater dangers to which the patients are exposed by the disease itself. Nor is it always possible to ascribe serious symptoms to the serum even when they follow closely on the treatment. Sudden death in epidemic meningitis is a not infrequent occurrence; and cases have even been reported in which death has taken place while the physician was engaged in getting ready to make the serum injection and even before lumbar puncture had been performed. The attempt to discredit the antimeningitis serum on the basis of an unfortunate experience seems, when everything is considered, hardly defensible.

MALARIAL PIGMENT (HEMATIN) AS AN ACTIVE FACTOR IN THE PRODUCTION OF THE BLOOD PICTURE OF MALARIA.*

By WADE H. BROWN, M.D.

(From the Pharmacological Laboratory of the University of Wisconsin, Madison, and the Pathological Laboratory of the University of North Carolina, Chapel Hill.)

In a recent article¹ the author submitted evidence to show that hematin intoxication in the rabbit was characterized by a paroxysm analogous to that of malaria, and a study of the effect of this pigment upon the blood is offered as further evidence of the rôle that hematin plays in malaria. While particular attention has here been directed to the formed elements of the blood, other important effects have been noted, such as the effect upon the coagulation time and the bleeding time.

The hematin, the hematin solutions, the doses, and the technique used in these experiments were identical with those used in previous experiments. In fact, many of the observations upon which this report is based were made in the course of experiments described in my former article. The experiments have been graded so as to determine the effect of a single injection of various amounts of alkaline hematin, and finally to follow the changes produced by injections repeated at various intervals over a period of days or even weeks. All phases of the experiments were controlled in two ways. One series of controls was made by injecting rabbits with the same amount of the solvent for the hematin (1 or 2 per cent. bicarbonate of soda dissolved in 0.85 per cent. sodium chloride solution) per kilo of body weight, and a second series of controls was made by first injecting the alkaline salt solution, and, after the

* Aided by a grant from The Rockefeller Institute for Medical Research. Received for publication, March 22, 1913.

¹ Brown, W. H., *Jour. Exper. Med.*, 1912, xv, 579.

animal's blood had returned to normal, injecting the hematin solution. This procedure was followed with only a few animals.

The Effect of Hematin upon the Red Blood Corpuscles.—The red blood corpuscles, although decidedly less subject to the toxic influence of hematin than either the leucocytes or the platelets, may be markedly reduced in numbers by the intravenous injection of hematin. A single injection of less than fifteen milligrams of hematin per kilo of body weight seldom produces any demonstrable effect upon the red cell count. If there is a decrease, regeneration is sufficiently rapid to obscure the change and this is probably the case. An injection of as much as twenty milligrams of hematin per kilo, however, usually shows, within a few hours, a decrease of as much as 1,000,000 cells, and frequently more. This decrease is still only transient although a reduction may be evident for as long as twenty-four hours.

The daily repetition of a dose of even ten milligrams of hematin per kilo will ultimately produce a definite anemia, and the larger the dose the more marked is the anemia. In well marked anemia the cells are irregular in size, the color is variable, and immature cells are present. The most common of these is the young basophilic cell, although nucleated red cells may be present in considerable numbers. Such an instance is illustrated by the following experiment:

Rabbit 5.—Albino, female; weight, 2.85 kilos. Within seven days this animal received eleven injections averaging 10 mg. of hematin per kilo of body weight. The red cells showed the following changes:

May 2, 1911. Red blood corpuscles, 7,596,000; nucleated red cells, 3 (normoblasts).

May 9. Red blood corpuscles, 3,200,000; nucleated red cells, 179; normoblasts, 153; intermediates, 21; megaloblasts, 5.

This experiment is cited merely to show to what degree the destruction of red cells can be pushed under favorable circumstances. The usual reduction, however, does not exceed one third of the normal count and nucleated cells are comparatively few.

As regards the hemoglobin, with single injections and slight reduction in the red cells, the hemoglobin index (Sahli) is not altered from the normal. With more pronounced destruction of red cells, and especially with the influx of immature cells into the circulation,

the hemoglobin index is usually reduced, but it may be normal or rarely increased above the normal. In some of these cases it is possible that hematin still in the circulation has influenced the hemoglobin determinations.

The anemia produced by the intravenous injection of alkaline hematin is due entirely to the hematin, as the alkaline salt solution has been found entirely incapable of producing the slightest degree of anemia, even when used in much larger amounts than were employed with the hematin. In fact, the only effect upon the red blood corpuscles, observed in the control animals, was in the opposite direction. Most of the animals showed no appreciable effect, while a few showed a very definite increase in the red cell count with a corresponding increase in the percentage of hemoglobin.

The mechanism of destruction of the red cells is by no means clear. *In vitro*, the alkaline hematin solution produces no hemolysis and this would be expected on account of the hypertonicity of the solution. In the animal body, on the contrary, some degree of hemolysis has been detected in many instances. The hemolysis, however, does not depend entirely upon the amount of hematin injected, as marked hemoglobinemia was noted in one case after an injection of ten milligrams of hematin per kilo of body weight, and was very slight or not demonstrable in several other instances where the dose of hematin was as much as twenty milligrams per kilo. The evidence at hand is not sufficient to warrant an expression of opinion as to the factors that determine the hemolytic action of hematin.

Microscopic study of the tissues shows that red cells are also destroyed intact as numbers of these cells are found included within phagocytic cells.

The Effect upon the Leucocytes.—The leucocytes, unlike the red cells, are markedly effected by simple alkaline salt solutions as well as by solutions of hematin, and this circumstance has added to the difficulties of establishing the leucocyte picture of hematin intoxication. The intravenous injection of alkaline salt solution produces a rapid decrease in the total number of leucocytes. Within three to five hours the count has again risen to normal, the rise continuing to a definite leucocytosis which lasts only a few hours and then

slowly falls to normal. A count taken twenty-four hours after the injection practically always shows a normal number of leucocytes. Differential counts (500 cells) show that all types of cells participate in these changes to some degree. The polymorphonuclear amphophile, however, is the cell that is most influenced. These cells show an immediate absolute reduction and frequently a relative reduction as well; they then increase rapidly so that their actual number may be well above the normal, while all other cells are still decreasing or at their lowest point; then follows a gradual fall to normal. Next to the polymorphonuclear cells, the large mononuclear and transitional group shows the most pronounced effect, an effect that is characterized by a rapid decrease with a slow recovery which continues well above the normal, both as regards percentage and actual numbers, but rarely exceeds 8 to 10 per cent. The small mononuclears decrease but slowly and regenerate even more slowly. The eosinophiles and basophiles are inconstant in their behavior, the former being but little affected while the latter may show marked fluctuations, characterized by an initial decrease and a marked subsequent increase.

By repeating the injections of alkaline salt solution daily, for a week or more, this picture is altered but little. The most important change is to be found in an increase of the mononuclear cells at the expense of the polymorphonuclears. The large mononuclears under these conditions may reach 12 to 14 per cent.

When the effect upon the leucocytes of a single dose of ten to fifteen milligrams of hematin per kilo is compared with the effect of an equivalent volume of alkaline salt solution, the differences that can be detected are but slight. To facilitate comparison the following abbreviated protocol and series of counts from one of my experiments are given.

Rabbit 48.—Belgian, male; weight, 2 kilos.

November 13, 1912. 9.30 A. M. White blood corpuscles, 6,600. Injected 6.6 c.c. of 2 per cent. sodium bicarbonate in 0.85 per cent. sodium chloride solution. The leucocytes were counted at intervals of one, three, five, and twenty-six hours.

November 20. 9.30 A. M. White blood corpuscles, 8,800. Injected 6.6 c.c. of hematin solution (10 mg. of hematin per kilo). The leucocytes were counted at intervals of one, three, five, and twenty-six hours. Complete counts are given in table I.

TABLE I.

The Leucocyte Reactions Produced by a Single Dose of Ten Milligrams of Hematin per Kilo of Body Weight and by a Corresponding Volume of Alkaline Salt Solution.

Time.	Leuco- cyte count.	Polymorpho- nuclear cells.		Small mono- nuclear cells.		Large mono- nuclear cells.		Eosino- philes.		Basophiles.	
		Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.
Nov. 13, 1912, 9.30 A.M.	6,600	54.0	3,564	32.8	2,164	6.4	422	0.8	52	6.0	396
10.30 A.M.	3,600	56.0	2,016	38.4	1,382	1.6	57	0.4	14	3.6	129
12.30 P.M.	5,800	82.0	4,756	15.6	904	2.0	116	0.0	0	0.4	23
2.30 P.M.	8,600	78.8	6,776	15.2	1,307	4.4	378	0.0	0	1.6	137
Nov. 14, 11.30 A.M.	6,600	57.2	3,775	17.4	1,148	9.4	620	0.4	26	15.6	1,029
Nov. 20, 9.30 A.M.	8,800	45.6	4,012	39.2	3,449	7.2	633	1.6	140	6.4	563
10.30 A.M.	5,800	25.5	1,479	71.0	4,118	2.5	145	1.0	58	0.0	0
12.30 P.M.	13,000	75.2	9,776	21.2	2,756	2.4	312	0.0	0	1.2	156
2.30 P.M.	9,200	77.6	7,139	17.2	1,582	3.6	331	0.4	36	1.2	110
Nov. 21, 11.30 A.M.	6,800	26.4	1,795	53.2	3,617	15.2	1,033	0.0	0	5.2	353

While many differences in the two series of counts in table I are apparent, the only feature to which I attach any importance is the relatively larger number of mononuclear cells, including large and small mononuclears, in the hematin series as compared with the control series at the end of the twenty-six hour periods. This difference has been practically constant under these conditions, but an almost equally marked increase in mononuclear cells may be encountered after repeated injections of alkaline salt solution over a period of several days. The reduction in the total leucocyte count from 8,800 to 6,800, as shown in the hematin counts, is misleading, as I have found an increase in the leucocyte count more common than a decrease. This feature of the hematin reaction will be considered later.

The production of a chronic intoxication by daily injections of small doses of hematin for a week or more brings out distinctly

the resemblance of the leucocyte picture of hematin intoxication to that of malaria. Following each dose of hematin there is a cycle of changes, such as is illustrated in table I, with the mononuclear cells dominating the picture more and more; especially is this true of the large mononuclears which gradually increase until they constitute from 18 to 25 per cent. of a leucocyte count that is normal, slightly increased, or rarely reduced. The tendency to a slight leucocytosis is still the one striking discrepancy between the two pictures. Pigmented macrophages are frequently present in large numbers and have been found in the blood as much as seventy-two hours after an injection of hematin. The polymorphonuclear leucocytes may also show phagocytosis although this is more common in extreme intoxications.

A typical example of the effect of chronic hematin intoxication upon the leucocytes is presented in table II, which contains the normal and final leucocyte counts made upon rabbit 15.

Rabbit 15.—Grey and white, male; weight, 1.6 kilos.

January 16, 1912. White blood corpuscles, 8,300.

This animal received 10 mg. of hematin per kilo of body weight on ten successive days.

January 27. White blood corpuscles, 11,800. The differential counts are shown in table II.

TABLE II.

The Leucocyte Reaction in Chronic Hematin Intoxication.

Time.	Leuco- cyte count.	Polymorpho- nuclear cells.		Small mono- nuclear cells.		Large mono- nuclear cells		Eosino- philes.		Basophiles.	
		Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.
Jan. 16, 1912, 8.30 A.M.	8,300	42.8	3,552	48.4	4,017	5.6	465	1.4	116	1.8	149
Jan. 27, 9.00 A.M.	11,800	23.8	2,808	50.2	5,924	22.2	2,620	0.4	47	3.4	401

If the conditions existing in pernicious forms of malaria be simulated by the injection of as much as twenty milligrams of hematin per kilo of body weight, and one or two injections be given daily,²

² Unless the animal is in the best condition it can not survive this dosage of hematin. The first injection will prove fatal to a few rabbits and the number of deaths will increase with successive injections, especially if the injections are given less than ten to twelve hours apart.

within a few days the animal will develop a marked leucocytosis. The polymorphonuclear amphophiles show a relative increase, the percentage of small mononuclears is decreased or normal, while the percentage of large mononuclears is normal or increased. Both the eosinophiles and basophiles are relatively decreased under these conditions. The most striking feature of the count is the high percentage of large mononuclear cells. While the percentage increase in these cells is not so great as in chronic intoxications it not infrequently reaches 12 or 14 per cent. of the total count. The normal and the final counts in an experiment of this character are given to illustrate this point.

Rabbit 19.—Grey, female; weight, 1.7 kilos.

March 18, 1912. White blood corpuscles, 11,400. Seven injections of 25 mg. of hematin, per kilo of body weight, were given between March 18 and 21, inclusive.

March 22. White blood corpuscles, 52,600.

TABLE III.

The Leucocyte Reaction in a Case of Extreme Hematin Intoxication.

Time.	Leuco- cyte count.	Polymorpho- nuclear cells.		Small mono- nuclear cells.		Large mono- nuclear cells.		Eosino- philes.		Basophiles.	
		Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.	Per cent.	No.
March 18, 9.00 A.M.	11,400	36.8	4,195	47.6	5,426	7.0	798	3.0	342	5.6	638
March 22, 9.00 A.M.	52,600	62.2	32,710	20.6	10,835	15.0	7,890	1.0	526	1.2	631

It will be noted that the increase in large mononuclear leucocytes, as shown in table III, is relatively greater than the increase in polymorphonuclear cells. This is not usually the case and is, therefore, to be regarded as an extreme example of the reaction of the large mononuclear leucocytes to hematin.

On reviewing the changes in the leucocytes induced by the injection of alkaline hematin, the increase in the total count of these cells with the lesser degrees of intoxication seems to be the feature most at variance with the leucocyte picture of malaria. A partial explanation of this difference is to be found in the fact that alkaline hematin is a strong local irritant and that it is extremely difficult

to inject into an ear vein, even in dilute solution, without exciting a local inflammatory reaction, while thrombosis of the vein is certain to follow a limited number of injections into the same vessel. Although all animals that showed any considerable inflammatory reaction have been discarded, it seems possible that with the strength of alkaline hematin that it has been necessary to use, the irritant action has not been without effect. The degree of this effect is impossible of estimation.

As regards the most characteristic feature of the leucocyte reaction,—the increase in large mononuclear cells,—a portion of this increase is certainly attributable to the alkaline salt solution used as the solvent for hematin, but, when this is deducted, I believe there is still a substantial margin that can be accounted for only by the action of the hematin.

Platelets, Coagulation, and Bleeding Time.—The most striking effect of hematin upon the blood is its destruction of the platelets and consequent prolongation of the bleeding time. While the normal number of platelets in the rabbit varies between rather wide limits, it is usually between 600,000 and 800,000, estimated by the method of Wright and Kinnicutt,³ and in a given animal it remains fairly constant from day to day. Following the injection of even a small dose of hematin, there is an immediate reduction in the number of platelets and the count continues to decrease for several hours. Large doses of hematin produce a correspondingly greater destruction of platelets which may exceed one half the total platelet count. If no further injections are given, the count will return to normal, or above, within twenty-four to forty-eight hours. Daily repetition of the injections of hematin, however, will greatly reduce the platelet count,—usually not lower than about one fourth of the normal count. A single dose of hematin sufficiently large to produce a greater reduction, or the repetition of smaller doses sufficiently often to accomplish the same result, are apt to prove fatal. In several instances, however, I have recorded greater reductions than this, the greatest being a reduction from 928,000 to 92,000. The series of counts in table IV is intended to illustrate the essential features of the effect of hematin upon the platelets as compared with the effect of alkaline salt solution.

³ Wright, J. H., and Kinnicutt, R., *Jour. Am. Med. Assn.*, 1911, lvi, 1457.

TABLE IV.

The Effect of Hematin upon the Platelet Count.

Time after injection.	No. 34. 25 mg. of hematin per kilo.	No. 32. An equivalent volume of alkaline salt solution.
Normal	616,000	560,000
1 hr.	192,000	598,000
3 hrs.	232,000	740,000
5 hrs.	156,000	646,000
24 hrs.	312,000	608,000
48 hrs.	480,000	594,000
75 hrs.	788,000	622,000

In connection with the destruction of the platelets, a tendency to persistent and even profuse hemorrhage is of frequent occurrence. A series of observations made on the coagulation time with the Boggs coagulometer and on the bleeding time by the method of Duke⁴ may be reported briefly. It was found that the ultimate effect of hematin was to prolong both the coagulation time and the bleeding time. The longest coagulation time recorded with the Boggs instrument was seventeen minutes, while eight to twelve minutes were not uncommon. In several instances, however, blood drawn in a test-tube remained permanently unclotted. The bleeding time is apparently much more affected. The ear vessels are usually abnormally constricted but if a normal degree of dilatation be procured profuse hemorrhage occurs from small cuts and bleeding continues for hours. In several extreme cases it was necessary to place a clamp on the ear after bleeding had continued two or three hours with no indication of cessation.

These phases of the action of hematin are apparently not influenced in the slightest degree by the presence of the alkaline salt solution. As regards the platelets, control experiments show almost uniformly an increase in the platelet count (table IV) after the injection of alkaline salt solution. Not a single instance of a definite decrease in the platelets has been observed. And no effect of any

⁴ Duke, W. W., *Jour. Am. Med. Assn.*, 1910, 1v, 1185.

kind has been noted upon either the coagulation or the bleeding time following the injection of alkaline salt solution.

DISCUSSION.

Comparing the various phases of the effect of alkaline hematin upon the blood of the rabbit with the blood in malarial fevers it can not be claimed that there is an absolute identity and yet there are no very great differences. The characteristic anemia of malaria has been ascribed to several accessory causes apart from the destruction of the red cells by the parasite developing within them. Prominent among these accessory causes has been the theory of a circulating toxin, destructive to the red cells. Hematin fulfills the essential conditions for such a circulating toxin, and it is only remarkable that this pigment should cause so pronounced an anemia as it has been found capable of producing. In this connection, it does not seem amiss again to call attention to the fact that the hemoglobin converted to hematin by the malarial parasite is not readily available for the regeneration of red cells, and the blood in this manner is rapidly depleted of hemoglobin iron. This condition undoubtedly contributes to the perpetuation of the malarial anemia.

Concerning the influence of hematin upon the leucocytes of malaria, one can speak with less assurance. In the first place, the possible normal variations of these cells in the rabbit are very great. Added to this is the influence of alkaline salt solution, which is difficult to estimate, and finally the difficulty of adjusting the dose of hematin so as to produce desired degrees of intoxication. Therefore only broad generalizations seem to be permissible. The leucocytosis of hematin poisoning is in sharp contrast to the leucopenia of the milder forms of malaria, but it coincides with the leucocytosis of the pernicious forms. The increase in the large mononuclear leucocytes, which is so characteristic of malarial blood, is also a prominent feature of the blood of hematin intoxication. Although the percentage of these cells is probably not as high as in malaria, this would hardly be expected as there are certainly other causes that contribute to the increase in large mononuclear leucocytes. Finally, the general cycle of changes produced by an injection of hematin is, at least qualitatively, analogous to changes occurring in the blood with the paroxysm of malaria. Altogether,

it would seem that hematin must play some part in the determination of the leucocyte picture of malaria.

Lastly, the destructive action of hematin upon the platelets and the consequent tendency to hemorrhage is not without a parallel in malaria. The platelets are probably the only bodies in the blood whose numbers in malaria are influenced solely by the circulating toxin of the disease, and it is of especial significance, therefore, that the platelets are reduced both in malaria and in hematin intoxication. The tendency to hemorrhage produced by hematin has its counterpart in the well recognized class of hemorrhagic cases of pernicious malaria. Here again the circulating hematin probably plays the important rôle in the production of the hemorrhagic condition. I do not wish to create the impression, however, that hemorrhages in malaria, or in hematin poisoning, are due solely to the destruction of platelets and prolongation of the bleeding time. This is certainly not the case in hematin poisoning in which profuse hemorrhage into the peritoneal cavity is of frequent occurrence in acutely fatal poisoning.

The pigment, hematin, must be regarded as an active factor in the production of many, if not all, of the important changes in the blood that characterize the various forms of malaria.

SUMMARY.

1. Intravenous injections of alkaline hematin in the rabbit produce an anemia the severity of which is proportional to the amount of hematin injected and the susceptibility of the animal.

2. Hemoglobinemia is an occasional consequence of hematin poisoning.

3. The leucocytes in hematin intoxication are usually increased in number and are always characterized by a high percentage of large mononuclear cells and by pigmented phagocytes.

4. The platelets are markedly reduced by alkaline hematin and ultimately a prolongation of the coagulation time of the blood and of the bleeding time results.

5. The anemia, the hemoglobinemia, the high percentage of large mononuclear leucocytes, the destruction of platelets, and the tendency to hemorrhage in malaria are all influenced by the malarial pigment, hematin.

THE EFFECT OF HEMATIN ON THE CIRCULATION AND RESPIRATION.*

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PLATES 2 TO 5.

In the course of investigations which one of us has been conducting upon the toxic manifestations of hematin when introduced into the animal circulation, the nature of the effects elicited with both sublethal and lethal doses of alkaline hematin was such as to indicate clearly that we were dealing with a substance that possessed a definite action upon the circulation and the respiration. Some phases of the toxic action of hematin in the rabbit have been described in detail in another paper,¹ and it is sufficient here to point out that the most characteristic clinical effect of the injection of small doses of hematin was found to be the production of a paroxysm similar to the malarial paroxysm in man. During the cold stage of this paroxysm, a marked constriction of the skin vessels occurs, seen especially in the ears, with a lowering of the surface temperature. This vasoconstriction gives place to an equally marked dilatation of the skin vessels and elevation of surface temperature with the onset of the hot stage of the paroxysm. It was further noted that, in those animals which succumbed to the toxic action of hematin within a short time after its administration, respiratory distress, marked prostration, extreme pallor, convulsions, and failure of the respiration were almost constant symptoms. In these animals there was usually found, post mortem, a pronounced splanchnic congestion with hemorrhage into the viscera and into the peritoneal cavity.

Accordingly, a study of the physiological action of hematin in

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¹ Brown, W. H., *Jour. Exper. Med.*, 1912, xv, 579.

alkaline solution offered a hope for a tangible explanation of these and other phenomena, many of which are characteristic alike of both hematin intoxication and human malaria.

The hematin used in these experiments was prepared from ox blood; the mode of preparation of the hematin and the alkaline solution was the same as that employed in experiments previously reported.² The solution used in all cases was formed of an 0.85 per cent. sodium chloride solution containing 2 per cent. sodium bicarbonate and either four or five milligrams of hematin per cubic centimeter. The effects obtained by the injection of this solution were controlled by injections of the same preparation of alkaline salt solution as that used in the preparation of the hematin solution. The injections were all made into the femoral vein.

Action on the Circulation.—The effect of hematin upon the blood pressure and upon the distribution of the blood in the body is very pronounced. Following the injection of doses of less than six milligrams of hematin per kilo of body weight in the cat or in the dog, a slight rise of the carotid pressure may occur. The injection of six to eighteen milligrams per kilo, however, produces a great fall of blood pressure in the cat which reaches a maximum in from one to five minutes and remains below normal from twelve to thirty minutes, or even longer in exceptional cases. In the dog the fall in pressure is usually not so great, but the duration of the effect is usually more prolonged than in the cat. The extent to which hematin may lower the blood pressure is shown in figures 1 and 2. Both of these tracings are from the cat and represent respectively effects of doses of eighteen and twenty-two milligrams of hematin per kilo of body weight.

On plethysmographing a loop of intestine and one hind leg simultaneously, and recording alterations in volume with piston recorders, it was found that prior to the fall of blood pressure there occurs a dilatation of the intestinal vessels which begins before the blood pressure starts to fall and continues for a long time. In one experiment the blood pressure remained below normal for about twenty

² Brown, W. H., *loc. cit.*

minutes, when other procedures interrupted the record. In several of the experiments the intestinal dilatation was of an extreme grade. During the fall of blood pressure the vessels of the leg constrict markedly. This compensatory action is not so perfect in the cat, in which the dilatation of the splanchnic vessels is less marked, and the fall of blood pressure is more marked than in the dog. In one experiment the cutaneous constriction started later than the intestinal dilatation, which would suggest that the constriction may be due, in part, at least, to the depletion of the cutaneous vessels to supply the engorged splanchnic vessels. We have concluded, however, that the cutaneous constriction is active for the reasons which follow.

In one experiment a slight rise of blood pressure followed the injection of hematin although the injection caused splanchnic dilatation. From our observations on the heart it is improbable that this rise of blood pressure was due to increased cardiac output, and in this case the constriction of the cutaneous vessels would seem to have more than compensated for the splanchnic dilatation. Unfortunately, there was no plethysmographic tracing from the leg in this experiment.

Figures 1 and 2 show typical tracings of the splanchnic dilatation and its relation to the blood pressure in the cat, while figure 3 shows plethysmographic tracings of the intestine and leg and the carotid pressure of the dog.

The splanchnic dilatation seems to be due almost entirely to the depression of the vasomotor center, since section of both the splanchnic nerves usually prevents entirely the dilatation and greatly reduces or entirely removes the blood pressure-lowering action of hematin. In fact, after cutting the splanchnics, we have noted a rise of blood pressure which further indicates that the cutaneous constriction is active rather than passive to the splanchnic engorgement. In one experiment a distinct but rather small splanchnic dilatation together with a very slight fall of blood pressure followed the injection of hematin after the splanchnics were cut. This single experiment indicates that a small fraction of the dilating effect of the drug is peripheral.

Action on the Heart.—The only definite effect of hematin upon the rate of heart-beat that we have noted is a slowing. In a few instances where doses of less than six milligrams of hematin per kilo of body weight were injected a transient acceleration of a few beats per minute occurred. Although the degree of retardation of the heart action is extremely variable, within certain limits it is proportional to the size of the dose of hematin. This is especially noticeable in the cat in which a dose of twelve to eighteen milligrams per kilo almost invariably produces a typical vagal pulse (figures 1 and 2). The same effect is less easily produced in the dog. As no slowing of the pulse rate occurs after the administration of atropin or section of the vagi, we may conclude that the slowing is due to stimulation of the cardio-inhibitory center.

An extreme irregularity in the amplitude and rhythm of the heart-beat occurred in many of our experiments on the cat but rarely in experiments on the dog (figure 2).

One experiment was performed with the heart in a plethysmograph and the changes in the heart volume were recorded by means of a piston recorder. The injection of hematin was followed by a marked loss of tone in both systole and diastole and a great decrease in the cardiac output. This reaches a maximum within about one minute and then the tone of the heart gradually returns until it becomes greater than the normal although the output remains diminished (figure 4). The heart, therefore, plays a part in the blood pressure-lowering action of hematin. The relative parts played by the splanchnic dilatation and the decreased cardiac output probably vary greatly in different experiments.

Action on the Respiration.—The primary effect of the large doses in which we employed hematin resulted frequently in an increased rate, but rarely in an increased amplitude of the respirations, a decreased amplitude being much more frequent. It seems probable from our records, however, that had we used smaller doses a stimulation of the respiration would have resulted. The larger doses of hematin produce marked depression of the respiration. In several of our experiments, especially on the cat, the respirations rapidly diminished in size until there occurred a complete cessation of the

respiration. This period of complete stoppage lasts thirty seconds or longer and is terminated spontaneously by very small respirations which gradually increase in size until the normal is reached. The rate is also slow, following the paralysis of the respiration, and this likewise increases gradually to the normal (figures 1 and 2). Death under hematin is due to paralysis of the respiration, the heart continuing to beat for some time after the respiration ceases.

CONCLUSIONS.

1. Doses of less than six milligrams of hematin and, under certain conditions, larger doses may cause a slight rise of blood pressure.
2. Large doses of hematin cause a profound and prolonged fall of blood pressure. The principal factor in this fall of blood pressure is the marked dilatation of the splanchnic vessels. The splanchnic dilatation either does not occur at all or but very slightly if the splanchnic nerves are cut. The splanchnic dilatation is partly compensated for by a marked constriction of cutaneous vessels and it seems probable that the cutaneous constriction is active and not simply passive to the splanchnic engorgement.
3. Hematin acts upon the cardio-inhibitory center causing a marked slowing of the rate of heart-beat, and in large doses produces a typical vagal pulse or even marked irregularities in the amplitude and rhythm of the pulse. Under hematin the heart at first shows great loss of tone but later the tone increases beyond the normal. The cardiac output for a time is greatly diminished.
4. In the large doses employed by us, hematin depresses the respiratory center and death under hematin is due to paralysis of this center.

EXPLANATION OF PLATES.

PLATE 2.

FIG. 1. Cat; weight, 2.8 kilos. Two injections of 5 c.c. of hematin (9 mg. of hematin per kilo) were given one and one half minutes apart. The second injection was given at 7. Read from left to right.

PLATE 3.

FIG. 2. Cat; weight, 3.4 kilos. Three injections of 5 c.c. of hematin (7.3 mg. of hematin per kilo) were given two and one half minutes apart, the third injection being given at 5. Read from left to right.

PLATE 4.

FIG. 3. Dog; weight, 11.5 kilos. Four injections of 3.5 mg. of hematin per kilo were given in rapid succession. Read from left to right.

PLATE 5.

FIG. 4. Cat; weight, 2.3 kilos. 5 c.c., or 8.5 mg. of hematin per kilo, were given at 2 and repeated at 3. Read from left to right.

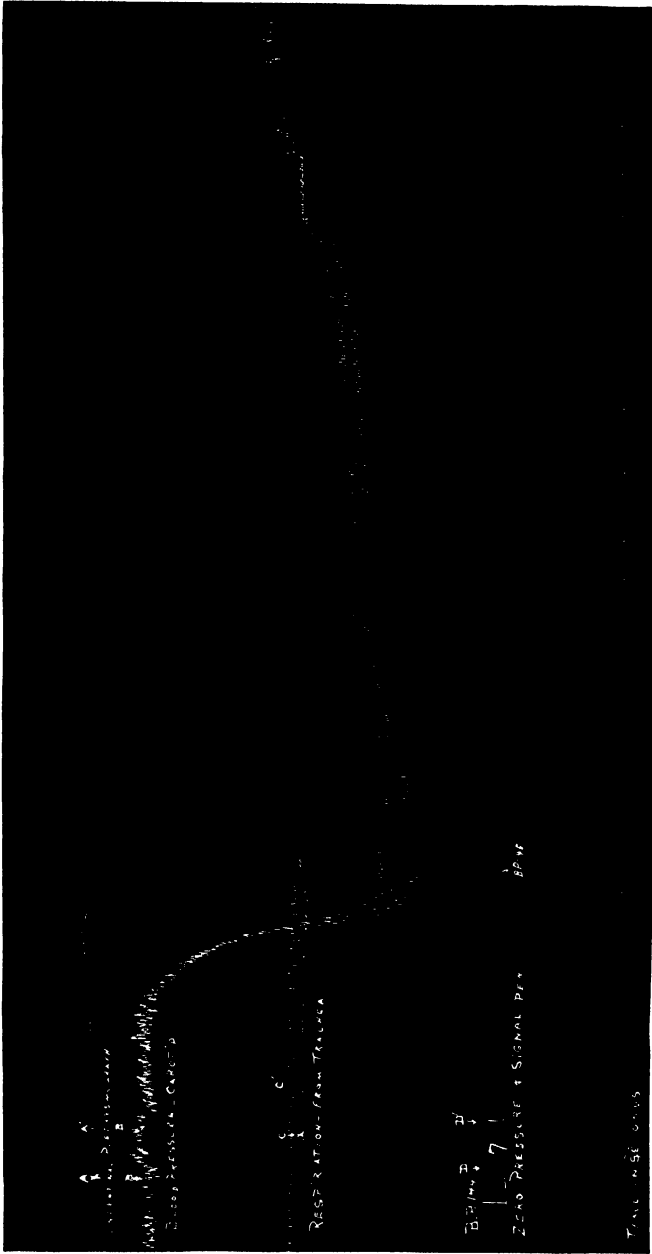


FIG. 1.

(Brown and Loevenhart : Effect of Hematin.)



FIG. 2.

(Brown and Loevenhart Effect of Hematin.)

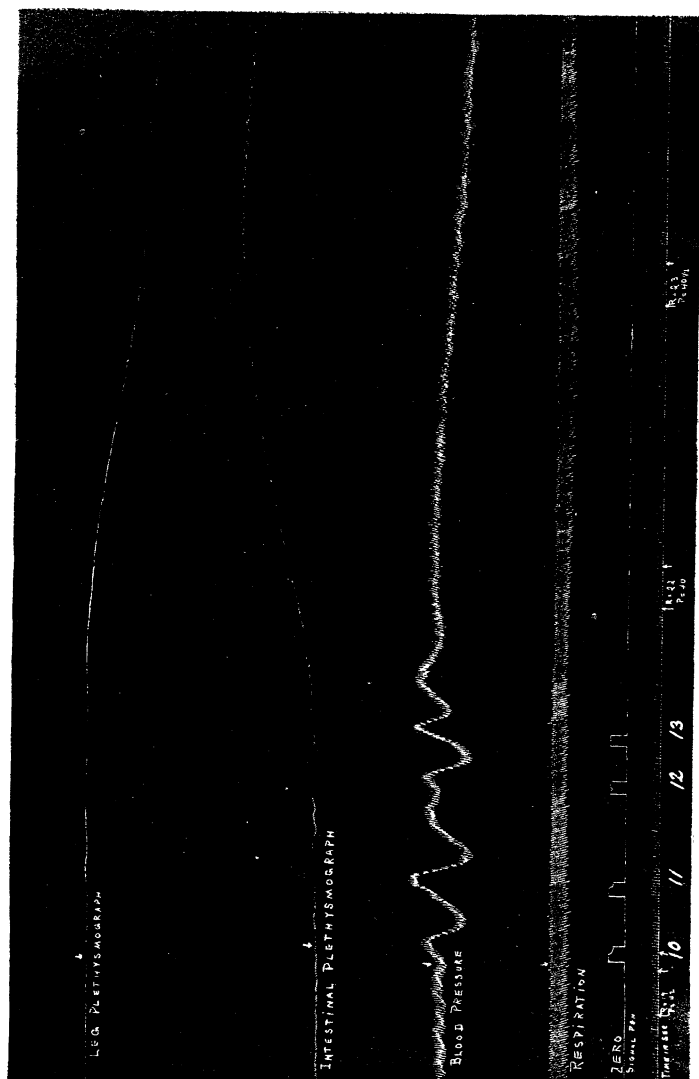


FIG. 3

Chosen and Lee, 1924, p. 11

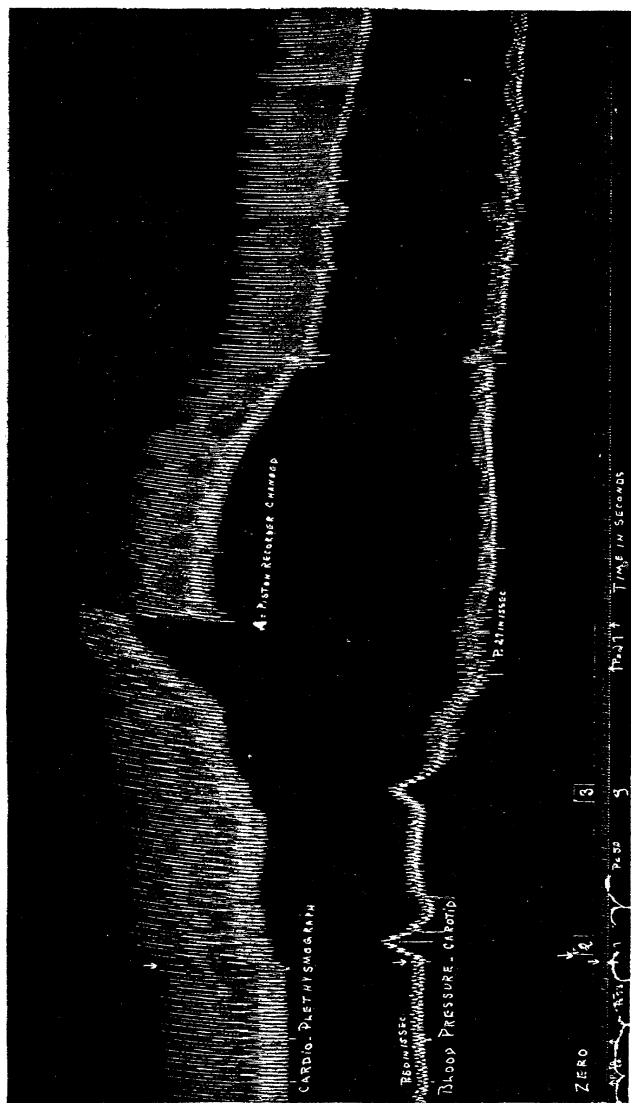


FIG. 4

(From Jan. Loewenhart: Effect of Hematin.)

THE TRANSMISSION OF *TREPONEMA PALLIDUM* FROM THE BRAINS OF PARETICS TO THE RABBIT.

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(From the Laboratories of The Rockefeller Institute for Medical Research,
New York.)

The finding of *Treponema pallidum*¹ in the substance of the brains of paretics has now been confirmed by several investigators in this country and abroad. Since the first report in which the *pallidum* was described as having been discovered in twelve of the seventy brains from which sections had been studied, I have now found the spirochetes in thirty-six of one hundred and thirty additional brains, from which sections stained by the modified Levaditi method² were examined. Thus, in two hundred cases of paresis so far studied the *pallidum* has been seen by me in forty-eight, or in nearly 25 per cent. of the specimens. I have, moreover, also succeeded in finding the micro-organism in one of twelve specimens of the spinal column, studied from cases of tabes dorsalis. A spiral organism morphologically identical with the *pallidum* was in this instance found in the posterior columns of the cord.

The findings already at hand, therefore, indicate unmistakably that *Treponema pallidum* plays an important part in the pathogenesis of general paralysis and possibly in tabes also. Since the demonstration of this micro-organism in sections, however, merely indicates its presence in relation to the pathologic lesions of these diseases, it becomes of great importance to ascertain from living examples something of the biologic properties of this organism, which remains so persistently within the central nervous organs in these two pathologic conditions. This is especially desirable since the conditions are so unlike the typical manifestations of syphilis, in which the *pallidum* is far less resistant.

1. Noguchi and Moore: A demonstration of *Treponema pallidum* in the brain in cases of general paralysis, Jour. Exper. Med., 1913, xvii, 232.

2. München. med. Wchnschr., 1913, lx, 737.

The question at once arises whether the spirochetes within the nervous organs are capable of producing lesions in animals similar to those caused by the *pallida* contained within the primary and secondary syphilitic lesions.

Through the courtesy of Dr. Rosanoff of King's Park State Hospital and Dr. Lambert of the Manhattan State Hospital, I obtained six fresh brains from parietic individuals. I succeeded in one of these in demonstrating in the fresh state the *pallidum* under the dark-field microscope. Rabbits were inoculated with emulsions prepared from the fresh brain tissues from the six cases.

The emulsions were injected intratesticularly with approximately 1 c. c. of the emulsions prepared in about ten volumes of citrate solution from pieces of brain tissue taken from various convolutions. Thirty-six rabbits were inoculated. With the exceptions about to be mentioned, the inoculations were followed by the usual evanescent inflammatory reaction, after which no further pathologic change was detected during a period of observation varying from three to six months.

From brain specimen 522, given me by Dr. Rosanoff, six rabbits were inoculated. The brain was obtained from a man 59 years old, who had been transferred, without previous history, to King's Park State Hospital on May 28, 1912. An abstract of the notes made on the case by Dr. Rosanoff is as follows:

The pupils were irregular; the left did not react at all; the right reacted very slightly to light; knee-jerks were absent, and the gait and station were both highly ataxic; the speech was slurred and the hands tremulous; grandiose ideas existed. A lumbar puncture on Jan. 5, 1913, yielded cerebrospinal fluid containing an excess of lymphocytes reacting positively, both to the butyric acid and the Ross-Jones test. Death took place on Feb. 16, 1913. The brain was found atrophied and the ependyma granular. Microscopically examined the brain showed the typical pial and cortical perivascular infiltration with lymphocytes and plasma cells. The immediate cause of death was bronchopneumonia.

Two of the six rabbits (Nos. 64 and 110), each of which received one cubic centimeter of the brain emulsion into each testicle on Feb. 17, 1913, gave the following histories:

Rabbit 64 developed an abscess in the left testis, which opened spontaneously on February 21 and then subsided, except for an induration which persisted. On March 25 exudate was withdrawn by means of a capillary pipet, and showed no *pallida*. At this time the Wassermann reaction was negative. On April 10 the

induration, which had persisted throughout, altered in appearance and now suggested a syphilitic lesion, but spirochetes could still not be demonstrated. The Wassermann reaction, however, was now weakly positive. On May 20 a nodule the size of a pea was noted in the parenchyma of the organ, but I was unable to find the *pallidum* in it. The Wassermann reaction was unchanged. On June 6 a typical chancre-like induration was present in the scar of the old abscess. A large number of *pallida* were now found under the dark-field microscope, and the Wassermann reaction had become strongly positive.

Rabbit 110 developed suppuration in both testes. The left opened on February 21, and the right on February 25. On March 15 both testes remained indurated. On March 25 the induration continued, but neither the *pallidum* nor Wassermann reaction was present. On April 10 the induration had taken on characters suggestive of a syphilitic lesion, but still no *pallida* were found. The Wassermann reaction had become weakly positive, however. On May 20 a distinct nodule appeared in the parenchyma of the right organ. While no *pallida* were found under the dark-field microscope, the excised tissue stained by the Levaditi method showed a small number of unmistakable morphology. The Wassermann reaction was unchanged.

DEDUCTIONS.

The results indicate that typical syphilitic scleroses containing *Treponema pallidum* have been produced in the testes of two rabbits by the inoculation of an emulsion of the brain obtained from a paretic individual.

The lesions developed slowly, requiring in the one instance ninety-two and in the other one hundred and five days. Taken all together the percentage of successful inoculations is thus far small, since they were obtained with one brain out of six. Both in respect to the slow development of the lesions and to the low percentage of infections the result is striking when contrasted with what occurs on inoculation of chancres, condylomata, or secondary syphilitic papules. By using several rabbits I have usually succeeded with every specimen of the latter, while I have been successful with only one specimen of brain out of six employed. The disparity is probably connected with the small number of *pallida* present in the brain tissue, although it may be due to a low degree of infectiousness of the brain spirochetes for the rabbit. The percentage (17), however, is about that (25) in which the *pallidum* has been detected by histologic methods in the brains of paretic individuals. A full report on this subject is to appear in a forthcoming number of the *Journal of Experimental Medicine*.

AN INVESTIGATION OF THE ORIGIN OF IMMUNE SERUM NECROSIS OF THE LIVER.*

By HOWARD T. KARSNER, M.D., AND JOSEPH C. AUB, A.B.

(From the Laboratory of Pathology (Phillips Fund) of the Harvard Medical School, Boston.)

Since the investigations into the nature of the cytotoxins have been pursued, much has been written of the effect of these bodies on organs in vivo. Delezenne¹ was the first to work with the so-called hepatotoxin, for which he claimed absolute organ affinity and by injection into living animals the production of a condition in the liver closely simulating that of phosphorous poisoning and of acute yellow atrophy. His animals did not develop icterus or hemoglobinemia, but showed leucin, tyrosin and occasionally glucose in the urine. Delezenne's findings were soon contradicted by Kraus,² who found icterus, hemoglobinemia, marked damming back of bile in the liver, but no primary injury of that organ. Fukuhara³ could not confirm the findings of Kraus in regard to "Gallenstauung," but did find small areas of necrosis. Pearce⁴ took up the matter and showed that immune sera formed by the injection of blood, bile, and of blood containing organs produced liver necrosis (with icterus and hemoglobinuria); "they (liver necroses) did not occur after the use of sera prepared by injecting urine, serum, and washed organs, except in one instance in which an exceedingly high dose of washed-liver serum was administered. The power of these various sera to produce conglutinative thrombi and consequently necrosis, varies naturally according to the degree of agglutinating activity which they possess." These studies were the first to suggest that the necrosis of the liver, following immune serum injections, is due to the agglutination or hyalin thrombi in the liver capillaries. The necrotic areas were described as being extensive, mid-zonal or periph-

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eral, often extending from lobule to lobule, associated with extensive hyalin thrombosis in the blood capillaries and showing complete hyalin necrosis of the affected liver cells, without fragmentation of the cell chains.

Previously, Boxmeyer⁵ had stated that some of the focal necrosis of the liver following experimental inoculation with hog cholera bacilli was due to plugging of the smaller veins and capillaries by hyalin thrombi and Flexner⁶ had shown that ricin, abrin, bacterial toxins and ether could produce such hyalin thrombi. It seems to be generally accepted that such hyalin thrombi are due to the agglutination and conglutination of red blood corpuscles. That plugging of the capillaries by various bodies could produce focal necrosis had been indicated by Mallory,⁷ who was able to produce focal necrosis experimentally by the injection into the liver of lycopodium, of carmine and of animal charcoal. He, however, appears to be a strong adherent of the toxic origin of necrosis as is indicated in a later study.

Of the more recent studies of liver necrosis, that of Loeb and Meyers⁸ has been of especial importance as regards the question as to whether simple obstruction or toxic action or both are of primary importance etiologically. These writers came to the conclusion that the earlier stages of ether necrosis of the liver are due primarily to direct action of the ether on the liver cells, the further extension of the necrosis being due to the obstructive influence of hyalin thrombi and of infiltrating leukocytes. This study has given rise to the thought that a somewhat similar attack of the problem of immune serum necrosis would be of value. Bearing in mind the possibility of agglutinative, anti-agglutinative, hemolytic, anti-hemolytic, complementary, anti-complementary activity of the materials used, the following series of experiments was followed out:

I. Intravenous injection of immune serum in living cats.—Animals killed immediately and tissues preserved in air, .85 per cent NaCl solution, fresh cat serum, inactive cat serum at both 0° C. and 37° C. Specimens removed at proper time intervals were fixed in Zenker's fluid and in formalin, embedded in paraffin, stained with hematoxylin and eosin. At 0° C., specimens were removed at six, twelve, twenty-four, and forty-eight hours. At 37° C., specimens

were removed at one, three, and six hours. Longer preservation at incubator temperature was impossible because of the cytolytic action of a bacillus in cats' livers morphologically like that described by Wolbach and Saiki⁹ in dogs' livers.

II. Perfusion of fresh livers with .85 per cent NaCl plus immune serum after washing by perfusion with .85 per cent NaCl. Preservation, etc., as in Series I.

III. Perfusion of fresh livers with .85 per cent NaCl plus immune serum plus fresh guinea-pig serum after washing by perfusion with .85 per cent NaCl. Preservation, etc., as in Series I.

IV. Perfusion of fresh livers with inactivated cat serum plus immune serum after washing by perfusion with .85 per cent NaCl. Preservation, etc., as in Series I.

V. Perfusion of fresh livers with fresh (active) cat serum plus immune serum after washing by perfusion with .85 per cent NaCl. Preservation, etc., as in Series I.

The immune sera were prepared by repeated injection into the rabbit of unwashed defibrinated cat's blood, either intraperitoneally or intravenously. All sera were titrated *in vitro* before being used and ranged from 1-90 to 1-400 in strength. The perfusion was done with from one hundred to two hundred cubic centimeters of fluid, depending on the size of the cat and immune serum was used in a dose sufficient to produce necrosis in the same weight cat if it were living. Guinea-pig serum was used in the proportion of one cubic centimeter to four hundred grams cat weight. All perfusions were done aseptically and the preservation was in aseptic containers and in aseptic fluids. The liver was cut into small pieces for preservation and was immediately fixed at the end of the proper time interval.

The controls showed that perfusion of salt solution alone is sufficient to produce well marked separation of the liver columns in the central zone. In these controls, very small areas of histolysis were found, usually near the center of the lobules. Those livers preserved at 0° C. frequently showed what appeared to be an increase in the amount of histolysis in Experiments IV. and V., but no definite mid-zonal or central area of extensive actual necrosis. The specimens preserved at 37° C. showed practically no change

except that in Experiments II., III., and IV. at the end of six hours numerous small areas of necrosis and fragmentation of cell columns as described by Wolbach and Saiki were found and in these areas large numbers of large rods (bacteria) were found. This change was found in the specimens regardless of the preserving fluid and from our studies no conclusion as to the relative value of these various fluids as preservatives can be drawn.

In these series of experiments nearly twenty cats were used and more than two hundred and fifty sections of livers examined histologically. It is not considered necessary to consume space with detailed reports of results that are negative.

From the results of these experiments it would appear at first glance that the direct action of the immune serum on the liver cells could be ruled out and that the cause of the necrosis is purely mechanical; but the type of necrosis produced by plugging of the capillaries by inert bodies as was done by Mallory and the necrosis produced by injecting agglutinated corpuscles as done by Pearce, is a granular necrosis as contrasted with a hyalin necrosis produced by the injection of immune serum into the living animal. Hence it is fair to conclude that the obstruction alone is not the only agent acting in the process and, as Pearce has already suggested, it is easily possible that the agglutinated corpuscles can give up, after lodging in the capillaries, sufficient amboceptor (cytolysin) to produce a serious effect on the liver cells. Furthermore, the mid-zonal position of the necrosis and also of the hyalin thrombi is indicative of an intimate connection between the two. Opie¹⁰ has shown that foreign bodies gaining access to the liver capillaries tend to lodge in the mid-zonal region provided the portal and hepatic circulations are otherwise intact. This accounts for the mid-zonal position of the agglutinated corpuscles and also of the hyalin necrosis, provided the connection between the two is accepted.

An attempt was made to place in the capillaries of the washed liver masses of agglutinated corpuscles. The corpuscles of five cubic centimeters autologous cat's blood were agglutinated and placed in the refrigerator for eighteen hours. At the end of this time the corpuscles were washed twice with salt solution, suspended in one hundred cubic centimeters salt solution and perfused through

the washed liver of the same cat, which was killed by bleeding immediately before the perfusion. No evidence of necrosis was found and the corpuscles were very rapidly dissolved during perfusion, probably because of the complement yielded by the liver cells. The reverse experiment also was tried. In this case the corpuscles were agglutinated in the incubator for forty minutes (one hundred cubic centimeters five per cent suspension) and then centrifugalized, the supernatant fluid being used for perfusion. Again no evidence of necrosis was found.

That the circulation in the areas of necrosis is not completely stopped is shown by the following experiment: A cat was given a large dose (one cubic centimeter to five hundred grams body weight) of a hemolysin capable of dissolving a five per cent washed corpuscle suspension in a dilution of 1-300. The animal was killed, the liver washed with one hundred cubic centimeters of .85 per cent sodium chloride solution, injected into the portal vein, and then a carmine gelatine injection mass injected under a pressure of twenty millimeters. At the end of the experiment the pressure was raised for two minutes to forty millimeters. The liver was removed, hardened in formalin at refrigerator temperature and frozen sections made which were stained with hematoxylin. The necrotic areas were easily visible and very thin strands of carmine could be seen penetrating into the capillaries between the cell chains. These strands of carmine were not of sufficient caliber to indicate that erythrocytes could pass through; but it seems certain that circulation of fluids could be regarded as easily possible. Hence the hyalin thrombosis is not to be considered as completely obstructive.

It has been shown that perfusion of immune serum is not in itself sufficient to produce liver necrosis under the restricted conditions of the experiment. The necrosis is not due to complete obstruction to circulation, but appears to be dependent on hyalin thrombosis. Experimental embolism does not produce a necrosis comparable to that following the immune serum hyalin thrombi. The hyalin thrombi are easily capable of yielding in limited areas a concentrated cytotoxin. Hence it is concluded that the immune serum necrosis is due partly to the hyalin thrombosis and probably in lesser degree to a cytotoxin liberated by the cells in the thrombus and

acting on the liver cells, a conclusion in support of the explanation offered by Pearce.

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AN EXPERIMENTAL STUDY OF THE LATE GLOMER- ULAR LESIONS CAUSED BY CROTALUS VENOM.*

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sity of Pennsylvania, Philadelphia.)

PLATE 6.

In 1909 I described acute glomerular lesions produced in the rabbit by the use of rattlesnake venom.¹ These lesions, which followed the intravenous injection of carefully graded doses of venom, were either hemorrhagic or exudative in character and involved either the tuft or the capsular space or both. The tubular epithelium was either normal or showed minor changes only. As it seems probable, in view of the work of Flexner and Noguchi,² that the selective action on the glomerulus is to be explained by the action of the endotheliolytic body of venom, the lesion was considered as essentially a vascular nephritis with severe endothelial changes, and therefore as one offering an opportunity for attempting the experimental production of a chronic nephritis. During the past few years this possibility has been kept in mind, and from time to time in connection with various other phases of the studies of experimental nephritis a more or less intermittent study has been made of the lesions occurring after various lengths of time and after single and multiple injections of venom, in the hope of demonstrating chronic glomerular lesions.

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¹ Pearce, R. M., The Production of Edema. An Experimental Study of the Relative Importance of Renal Injury, Vascular Injury and Plethoric Hydremia, *Arch. Int. Med.*, 1909, iii, 422; An Experimental Glomerular Lesion Caused by Venom (*Crotalus adamanteus*), *Jour. Exper. Med.*, 1909, xi, 532.

² Flexner, S., and Noguchi, H., The Constitution of Snake Venom and Snake Sera, *Univ. Penn. Med. Bull.*, 1902-03, xv, 345.

The venom used in the experiments was the dried venom of *Crotalus adamanteus*.³ It was dissolved in salt solution in the proportion of 0.25 of a milligram to one cubic centimeter, which was the usual initial dose. In the first series of animals fresh venom only was used, but as this frequently caused a rapidly fatal result, an old solution of venom which had been kept in the laboratory for a year was substituted. The older solution had lost much of its lethal power, that is, its neurotoxic and hemolytic powers were decreased, but its endotheliolytic power, as shown by the occurrence of albuminuria and the presence of exudation in the capsular space, had not been impaired. It was therefore used in the earlier treatment in doses of 0.25 or 0.5 of a milligram, rising gradually to two milligrams, and then followed by doses of 0.5 of a milligram of fresh venom at various intervals. The intervals between injections depended on the general condition of the animal and the amount of albuminuria. The initial injection was always followed by albuminuria and occasionally by hematuria or hemoglobinuria. When the albumin disappeared or had decreased greatly in amount, another injection was made, and as the periods of albuminuria lessened, the doses were increased. As sometimes a single injection caused a persistent albuminuria for weeks, while in other animals four to eight injections were given, the entire series represents the results of single and repeated injections. Despite the fact that some animals showed a tendency to immunization, it was impossible, even with the greatest care as to food and environment, to carry an experiment over a period of more than six to seven weeks. No cause could be found for this other than a progressive emaciation presumably dependent on the chronic intoxication.⁴

The results are based on the study of twenty-four rabbits, all of which received venom by intravenous injection.

Five animals died within four days after a single injection. Of these, one, which succumbed in six hours, presented the hemorrhagic, and four the typical exudative glomerular lesion of acute

³ This venom was obtained through the kindness of Dr. Noguchi, of The Rockefeller Institute for Medical Research.

⁴ In some instances the loss of weight was equal to 40 to 50 per cent. of the original weight, though usually it was about 25 per cent.

poisoning. One of the latter, dying on the third day, showed also an extreme degree of hyaline degeneration of the epithelium of the convoluted tubules.

Two animals died on the tenth day, after second and third doses respectively, with the usual acute lesions and no evidence of a reparative process. In each instance death followed an increase in the dose. Four died between the fourteenth and twentieth days. These represent one, two (2), and five injections; and all but one (representing two injections) showed evidence of repair of the glomerular lesion. The details of the experiments follow.

Experiment 2. Eighteenth Day Lesion; Multiple Injections.—A rabbit weighing 1,560 gm. received 0.5 mg. of venom (old solution) in the ear vein on October 2 and 4, and 1 mg. on October 6. As the injections caused little albuminuria the treatment was continued by using 0.5 mg. of fresh venom on October 11, 13, and 15. The last injections caused a pronounced albuminuria with hematuria. The animal died on October 19. Its weight was 950 gm. No gross lesions were found at autopsy.

Histological Examination.—The kidneys show well marked hemorrhagic and exudative lesions in the glomeruli; hyaline, granular, blood, and hemoglobin casts in both convoluted and collecting tubules; and granular degeneration of the epithelium of the convoluted tubules and loops of Henle.

In addition, however, to these acute lesions, the kidney shows distinct evidence of an attempt to repair the glomerular injury. Here and there it is possible to observe a penetration of the cells of the compressed glomerular tuft into the mass of hemorrhage lying either in the tuft itself or in the capsular space; less rarely is this seen when the capsule contains only serum and fibrin. The cells derived from the endothelium of the tuft are, for the most part, oval or elongated, with large deeply stained nuclei and scanty protoplasm which tends to take the basic stain. Despite the dense staining of the nuclei, a vesicular arrangement is frequently evident. Sometimes the cells, instead of penetrating the mass of hemorrhage, form a capsule about it, suggesting by their appearance, when they surround small masses, a giant cell formation. This proliferation is always from the tuft, never from the capsule (figure 1).

Experiment 7. Sixteenth Day Lesion; Multiple Injections.—A rabbit weighing 1,420 gm. received 1 mg. of venom (old solution) on October 4 and 6. The injections caused only a transient albuminuria, and therefore 0.5 mg. of fresh venom was given on October 11. This caused an albuminuria, increasing daily

in severity, with death on October 20. The weight of the animal after death was 870 gm.

Histological Examination.—The same lesions are found as in experiment 2, but with less marked evidence of glomerular proliferation.

Experiment 17. Eighteenth Day Lesion; Single Injection.—A rabbit received 0.25 mg. of fresh venom on October 26. This caused well marked albuminuria which persisted until death on November 14. Many of the glomerular tufts were compressed by hemorrhage into the capsule or distended by hemorrhage in the center of the tuft itself.

Histological Examination.—The cells of the compressed tufts show marked proliferation with a tendency for the new cells to invade the masses of hemorrhage. No typical mitotic figures are seen, but the character of both the nucleus and protoplasm indicates that these are young cells, and occasionally irregular masses of nuclear material suggest degenerated mitoses.

The period of twenty to thirty days is represented by three animals. Of these one, weighing 1,280 grams, died on the twenty-fourth day after a persistent albuminuria and a loss in weight of 520 grams. The injections were 0.5 of a milligram of venom on October 2 and 4, and one milligram on October 6, with death on October 26.

Microscopic examination shows that the acute lesions had subsided, the only evidence of their earlier presence being the presence of occasional casts and compressed masses of red cells in the glomerular spaces and tufts. About these masses definite proliferation of endothelial cells can be seen, and not infrequently endothelial cells are seen penetrating the hemorrhagic masses. Although no mitotic figures can be clearly distinguished, many of the compressed glomeruli appear to have an increased number of nuclei, and recall the glomeruli of subacute intracapillary glomerular nephritis as seen in man.

Of the other two animals of this group, one, receiving a single injection of 0.25 of a milligram of venom, died after twenty-nine days, and the other, receiving several injections, after twenty-six days. Albuminuria was persistent and marked in each case. Histological examination shows extensive granular degeneration of the convoluted tubules and many casts, but no glomerular lesions.

Seven animals survived for a period of one month or more. Two of these represent a single injection; the others, two to seven injections. The longest period of survival was thirty-eight days.

Albuminuria was persistent in all until death, but although all showed degenerative changes in the tubular epithelium, in none were glomerular changes prominent, and in only two were they even suggestive of reparative proliferation.

These observations indicate that in venom intoxication terminating in death at the end of five to six weeks the early acute glomerular lesion does not tend to become chronic; that is, it does not become transformed through proliferation and reparative processes into a subacute or chronic glomerulonephritis. Proliferation in the presence of an exudative (serum and fibrin) lesion was not seen; in the hemorrhagic lesion, proliferation of the endothelial cells of the tuft and the penetration of these cells into the masses of hemorrhage occurs, but this process is analogous to the organization of a red thrombus and is therefore not similar to the progressive proliferation of tuft or capsule seen in man. The attempt to produce, through the use of venom, a chronic glomerular lesion as the result of repair of acute lesions therefore fails, and with this failure disappears also the possibility of demonstrating experimentally that chronic glomerular lesions in man may have as their origin an injury caused by an endotheliolytic substance.

Several attempts to produce chronic lesions in the dog by repeated injections have likewise been unsatisfactory, owing to the fact that the toxic effect and the degree of hemolysis in this animal are much greater than in the rabbit. As the result of the study of the kidneys of five dogs receiving venom, it may be said that the lesion produced by small doses (0.25 to 0.5 of a milligram per kilo) is a moderate grade of degeneration of the tubular epithelium with cast formation. After the administration of larger amounts this degeneration is more severe, and to it is added the evidence of hemoglobin destruction; but seldom does one see in the glomerular capsule the exudate of serum, and never that of fibrin and red blood corpuscles, which is characteristic of the action of this poison on the rabbit's kidney. On the other hand, a peculiar hyaline change in the capillary loops of the glomerular tuft is a frequent lesion.

In no instance was it found possible to carry a dog for a long period of time. In attempts to accomplish this the smallest dose capable of causing albuminuria was administered, and this dose was

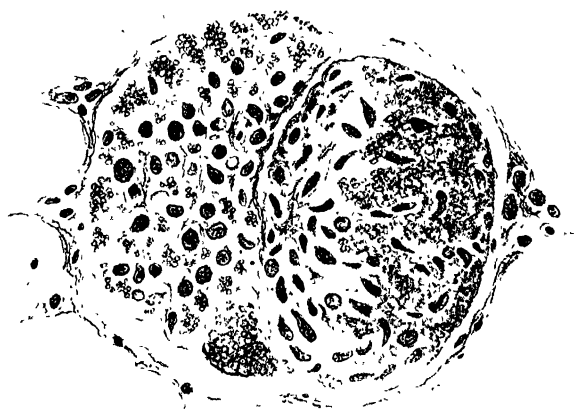


FIG. 1

(Pearce: Lesions Caused by Crotalus Venom.)

not given again until the albuminuria had disappeared. Despite this, it was not possible to carry an animal longer than two weeks, and at no time could evidence of reparative or subacute glomerular lesions be found.

SUMMARY.

The acute exudative glomerular lesion of the rabbit's kidney caused by crotalus venom does not lead to a subacute or chronic glomerulonephritis. The hemorrhagic lesion of the glomerular tuft may show a process of repair characterized by the ingrowth, into the hemorrhagic masses, of endothelial cells from the uninjured part of the tuft. This process is, however, more analogous to the organization of a red thrombus than it is to any form of glomerular lesion known in man, and can hardly serve as an experimental demonstration of the mode of development of a subacute or chronic glomerular nephritis.

On the other hand, crotalus venom causes a persistent albuminuria and extensive tubular degeneration and cast formation, with death, preceded by great emaciation, after five to six weeks.

EXPLANATION OF PLATE 6.

FIG. 1. Experiment 2. The glomerulus of a rabbit that died on the eighteenth day after repeated injections of crotalus venom. One half of the glomerulus is uninjured and is seen at the left of the figure; in the other half the hyaline area of an early hemorrhage is seen to be invaded by endothelial cells. Leitz oil immersion lens $\times 17$; ocular 4.

THE GROWTH OF TISSUE IN ACID MEDIA.*

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PLATE 7.

It has often been shown that the higher animals are extremely sensitive to changes in the reaction of their circulating fluids, and that if the blood be made acid death results. There seems to be a general belief that the cells themselves are unable to withstand an acid reaction of the medium about them. The truth of this belief can now be tested by the use of tissues growing *in vitro*.

In the course of some work with the cells of a chicken sarcoma proliferating *in vitro* I was led to add blue litmus to the plasma medium, and observed that a marked acid change accompanied cell growth. All the tissue fragments were at first stained blue and those which remained inert did not lose this color; but the others which were destined to proliferate became violet or pink within a few hours, often indeed before any sprouting out of cells was observable; and any considerable degree of growth always resulted in a focal pink change in the medium. The mere elaboration of acid by growing tissues is in itself no cause for remark. But the reaction with litmus means that a considerable amount of acid is produced, since this indicator when in plasma is relatively insensitive, owing to the high combining power for acid of the proteins. And that the cells should be capable of further active growth in the medium which they have rendered acid deserves attention and calls for further observations.

Chicken plasma, chicken sarcoma, and the heart muscle and large blood vessels of embryo chicks have been used for the present experiments. The ordinary, hanging-drop plasma preparation was found

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to be unsuitable since it presents too thin a layer for the proper observation of color changes. Instead small Petrie dishes have been used and the tissue bits distributed in a layer of plasma one to two millimeters thick. With pure azolitmin the results are not good, and with pure litmus (Merck's reagent) they are no better than with the watery solution obtained from Kahlbaum's cube litmus and used for tinting litmus milk. This solution as prepared by adding 250 cubic centimeters of water to 500 grams of litmus, heating to 80° C. for one hour, and filtering, is distinctly though only slightly alkaline. It contains numerous impurities including carbonates. When added to chicken plasma, as yet unclotted, in the proportion of one part to ten it colors the medium a deep blue. Such a medium is better than a violet one because the color changes are sharper.

The findings with the chicken sarcoma and with non-neoplastic connective tissue differ only in intensity. The production of acid by the chicken sarcoma in quantities sufficient to change the reaction of the medium is prompt and marked, as is the sarcoma's growth. In one instance where eight or ten small fragments of the tumor grew in a dish with 0.8 of a cubic centimeter of plasma mixture, the diffuse pink change in the medium at the end of two days' incubation was found by test to be equivalent to that brought about in a similar bulk of the unclotted medium by 0.12 of a cubic centimeter of N/10 hydrochloric acid. Fragments of tumor or of late embryos (twenty days) placed in the tinted plasma are all at first colored blue, and only those which start to grow show the change to violet and pink. The color differences between active and inert tissue bits are very striking in dishes where some fragments grow and others perish (figure 1). They constitute a good macroscopic index to proliferation. The growing tissue itself appears as a whitish, unstained zone, or halo, in a pink medium. Material from very young embryos, which survives practically *in toto* the transfer to the plasma medium, likewise remains unstained, and some of the visceral tissues from such a source appear to affect the reaction of the plasma medium very little in their growth.

Tissues grow excellently in a medium tinted with methyl orange, but the acids they produce do not affect this indicator,—presumably because they are organic. With congo red a slight violet change is

sometimes observed in the zone of proliferation, but the reaction is only definite in the case of cells into which the stain has penetrated. These are practically always cells distended with fat droplets and far gone in degeneration. Their cytoplasm may be colored a bright violet. Dead tissue fragments stain red usually, but growing tissue is unaffected by the stain and is seen as a pale halo in the midst of the red medium. The occasional violet tinting of isolated cells cannot be due to free carbon dioxide, for though congo red reacts to it in water this does not happen when the gas is bubbled through unclotted plasma tinted with the indicator.

A striking fact with regard to the elaboration of acid in the plasma medium, and one which must be of much importance for tissue cultures is the small extent to which it diffuses. It is relatively unaffected, as regards neutralization, by the amount of plasma beyond the zone of growth, and this even when the plasma web has been thinned by dilution. When growth is stopped by placing the preparation in the ice-chest, and alkaline plasma is present beyond the zones of growth, neutralization gradually occurs in the latter, but so slowly that it may be incomplete at the end of forty-eight hours. Under conditions *in vitro* which do not permit of an artificial circulation the growing tissue finds itself almost at once in an acid medium. When growth is prolonged by washing the tissue in Ringer solution and reimplanting it in fresh plasma, its alkaline reaction is temporarily restored, only to disappear as growth begins anew. The more rapid the growth of a tissue *in vitro* the less are the opportunities for its products of metabolism to be diluted by diffusion, and so the more unfavorable the conditions under which its growth must continue. This circumstance probably accounts to a considerable degree for the difficulties which Lambert¹ has experienced in keeping rat tumor alive *in vitro*. Similar difficulties are experienced with the chicken sarcoma.² When first implanted in plasma it grows almost explosively and produces markedly acid foci; but reimplanted it usually fails to grow.

No attempt has been made to determine the nature of the acids

¹ Lambert, R. A., *Jour. Exper. Med.*, 1913, xvii, 499.

² Carrel, A., *Jour. Exper. Med.*, 1912, xv, 516.



FIG 1.

(Rous' Growth of Tissue in Acid Media.)

elaborated during growth *in vitro*. Carbonic and lactic acids are presumably present in greatest amount.

SUMMARY.

Connective tissue cells of chick embryos and cells of a chicken sarcoma, proliferating *in vitro*, soon render acid the plasma about them, but they nevertheless continue to grow well. Evidently the tissue cell will withstand a considerably greater change in the reaction of the fluids about it than has usually been supposed.

Under conditions of *in vitro* life in plasma, which do not provide for an artificial circulation, the acid produced by growing tissues diffuses only slowly and is subject to little dilution from this source. About tissues which grow very rapidly *in vitro*, as, for example, tumor tissues, there must be a marked concentration of metabolic products, and this may largely account for the poor results of attempts at the continuous propagation of such tissues *in vitro*.

EXPLANATION OF PLATE 7.

FIG. 1. Chicken sarcoma in litmus plasma; twenty-four hours' incubation. A pink change indicates tissue growth. The tumor fragments remaining inert are stained blue. Two color photograph; preparation one third larger than natural size.

ADDITIONAL STUDIES ON THE PRESENCE OF SPIRO- CHÆTA PALLIDA IN GENERAL PARALYSIS AND TABES DORSALIS.*

By H. NOGUCHI, M.D.

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New York.)*

The majority of clinicians of the present day are agreed in admitting that general paralysis and tabes dorsalis are in their origins intimately connected with a previous luetic infection. It was, indeed, through the untiring and systematic studies of numerous investigators, notably of Esmarch and Jessen, Kjelberg, Fournier, Erb, Winge and Sandberg, Sternberg, Althaus, Virchow, Krafft-Ebing, Kraepelin, Gowers, Möbius, Strümpell, Raymonds, Binswanger, Leredde, Hallopeau, Homén, Mendel, Nonne, Mott and Collins, that the existence of an undeniable relation between syphilis and the so-called parasyphilitic affections of the central nervous system was definitely established. The theory that parasyphilitic conditions are in some way connected with a previous syphilitic infection is based chiefly upon the fact that the majority of individuals suffering from general paralysis or tabes dorsalis have had syphilis in early life. This argument, however, has been met by a great deal of opposition, on the ground that neither general paralysis nor tabes dorsalis show the characteristic histopathological lesions of syphilis and that anti-syphilitic treatment is of no avail in these diseases. Had we not had cases of syphilis of the central nervous system, which were typical from the histopathological as well as from the therapeutic standpoint, we might have assumed that syphilis of the brain and spinal cord presents special features not found in other organs. But as we are also confronted with typical cases

* Read by invitation before the Dermatological Section of the New York Academy of Medicine, March 4, 1913, with a demonstration of lantern slides and microscopical specimens.

of cerebral and cerebrospinal syphilis, it becomes difficult to entertain the theory that general paralysis and tabes dorsalis constitute real syphilitic affections. Fournier and others created a conventional classification under the name parasyphilis or metasyphilis, while others again have proposed to call these conditions postsyphilitic. It is true that certain authors have advanced the theory that general paralysis and tabes dorsalis are the consequences of the deleterious effect of some toxic substances emanating from some as yet unknown metabolic anomaly brought about by a previous syphilitic infection. In this case one would have to deal with the after-effects and not with the inciting agent of the infection; hence no benefit would be attained by any treatment that is directed against the virus itself.

The question whether or not in so-called parasyphilitic or postsyphilitic affections of the central nervous system we are to deal with the living virus or with the effects of post-infection unaccompanied by the living virus, is not merely of theoretic interest, but of grave practical importance, since upon this one point must depend the nature of the therapeutic measures to be adopted.

Since Schaudinn and Hoffmann made the momentous discovery of the presence of *Spirochæta pallida* in all stages of syphilis, many eminent pathologists have made innumerable efforts to demonstrate the presence of the syphilis organism in the nervous tissues in cases of so-called parasyphilitic affections, but without success. This fact in itself, however, is not sufficient to prove the absence of the *pallida*, for the presence of the positive Wassermann-Neisser-Bruck reaction in the blood and liquor cerebrospinalis, the pleocytosis and globulin increase in the spinal fluid, as well as the steadily progressing morbid processes of the patients, all point to the probability of the virus being present in the affected organs. Besides, there are many instances of tabes incompleta, in which an adequate and thorough administration of salvarsan or neosalvarsan has led to definite subjective as well as physical improvement.

My interest in the question of parasyphilis was first aroused when my studies chanced to lead me into the field of syphilitic research, but I did not take up the matter seriously until my attention was drawn to a peculiar phenomenon which I noticed during my

studies on the cultural properties of *Spirochæta pallida*. I discovered that under certain conditions and when grown in pure cultures, the *Spirochæta pallida* assumes the form of minute granules, from which, upon transplantation into a suitable medium, spiral forms are seen to sprout. This observation suggested to me the possibility of the *Spirochæta pallida* being present in a granular form in cases of parasyphilis. I assumed, therefore, that the failure of so many eminent investigators to demonstrate the presence of the *Spirochæta pallida* in the brains of paretics might be due to the absence of the spiral forms of this organism. With this idea in mind I began to examine a number of specimens of brains from cases of general paralysis. As I and Dr. Moore have reported elsewhere, I succeeded in a short time in demonstrating the typical spiral forms of the *pallida* in 12 out of the 70 cases comprising the first series of cases. This rather unexpected finding made it desirable to pursue the subject still farther in order to clear up the mystery as to why the organisms had not been perceived ere this. It is true that in many specimens it is extremely tedious and often impossible to detect the *pallida*, and I intend, therefore, on this occasion to deal with my further experiences in connection with this subject, describing, in particular, some technical points which facilitate the discovery of the microorganism in the tissues of general paralysis and tabes dorsalis. I shall then report the results hitherto obtained by me.

Up to the present I have examined 200 specimens of brains from cases of general paralysis and 12 specimens of spinal cords from cases of tabes dorsalis, all of which were placed at my disposal through the courtesy of Dr. Cotton, Dr. Dunlap, Dr. Elser, Prof. Ewing, Dr. Fisher, Prof. Graves, Dr. Hough, Dr. Kaplan, Dr. Lambert, Dr. Moore, Captain Nichols, Dr. Orton, Dr. Rosanoff, Dr. Smith, Prof. Spiller, Prof. Weisenburg and Dr. Zabliski, to whom I here wish to acknowledge my indebtedness.

The specimens were stained according to Levaditi's method, with certain modifications to be mentioned later, although it should be stated that this method in some instances produces quite satisfactory results. In the total 200 specimens of parietic brains, 48 cases were seen to possess the *Spirochæta pallida*. The ages of the patients showing the microorganism varied from 29 to 75 years; in one

case where the pallidæ were found, the patient had had the illness as long as six years. The *Spirochæta pallida* was also demonstrated under the dark-field microscope in one of the six fresh parietic brains given me by Drs. Lambert and Rosanoff. This specimen contained very few pallidæ, and in the stained preparation I failed to locate them.

Unlike the brain specimens, great difficulties are encountered in the examination of the spinal cord. In my early attempts I prepared transverse sections, but was considerably hampered in my search for the microorganisms by the presence of numerous neuroglia fibres and cross sections of the nerves. By cutting the tissue longitudinally this difficulty was greatly lessened and I was able to find the *Spirochæta pallida* in the posterior column of the dorsal cord in one of the 12 specimens. The organisms were extremely sparsely distributed and much patience was required before one was discovered. The other specimens are still under continued observation and I intend at a later date to report more definitely upon the relation of the pallida to the lesions.

DISTRIBUTION OF SPIROCHÆTA PALLIDA IN PARETIC BRAINS AND THEIR RELATION TO THE TISSUE ELEMENTS.

I am not in a position to discuss at length the question of the distribution of the organisms, as it was not my intention to investigate this phase of the problem. I can only state that the gyrus frontalis, gyrus rectus and Roland's region in particular were selected for the present study and gave the results above reported. In several instances the gyrus hippocampi, Ammon's horn and other regions were also examined and in one case the spirochætæ were found in all these parts, being, however, much less numerous there than in the motor region.

Spirochætæ pallidæ are found more frequently and in greater quantities in the cortical layers than in the nerve-fibre zone. In some sections they are found in groups of varying sizes, scattered irregularly among the nerve cells and neuroglia fibres; in others isolated, widely scattered examples of the organism can be detected. By counterstaining the sections with toluidin blue or thionin, it is seen that a pyramidal cell is closely surrounded by one or more spiro-

chætæ, and in some instances a part of the pallida may be seen to have become inserted into the cytoplasma of the nerve cell. Some organisms lie in the perineural spaces along the axis-cylinder processes and certain irregular, amorphous precipitates are often seen in the vicinity of such cells, these being probably of the nature of an exudate. The nerve-cells infested by the pallidæ show the degenerative changes as indicated by their modified contour, distortion and swelling, or by the disappearance of the nucleus as well as of the processes. The pallida is very rarely found in the vicinity of the blood vessels and almost never within the vessel walls. In the pia mater I have not been able to find the pallida with certainty.

TECHNIQUE.

Before proceeding to describe a method which I found was productive of more satisfactory results than those hitherto obtained, I must again emphasize that the old Levaditi method has often given good results. It seems, however, that in such instances the original technique was not strictly followed out and the thickness of the tissues, in particular, greatly exceeded that which had been recommended as necessary. Thus, it is often useful to take a piece of parietal brain 5 mm or more thick, instead of 2 mm, because in such tissue a less deeply impregnated area is obtained within the interior portion in which the pallida is seen to stand out in sharp distinction from the less deeply impregnated neuroglia fibrils.

Another extremely important point which has not been strictly respected by many is the fact that while the neuroglia fibrils stain well in an imperfectly fixed tissue, *Spirochætæ pallidæ* never take on the silver impregnation, regardless of the time and temperature in which they are exposed to it. Thus, it is of fundamental importance, in order to ensure success, that the tissue be completely fixed prior to its impregnation.

It is also of fundamental importance to know that the prolonged action of formalin interferes with the staining of the neuroglia fibrils, while it accelerates that of the pallida; thus, specimens which have been preserved in formalin at least one year give the best results, although recent specimens have also in many cases proved successful.

With the above facts as a basis I have formulated the following method, which I have found to be quite satisfactory:

From a specimen taken from the gyri frontali, gyri recti or any other region and hardened in 10% formalin, a slice of tissue measuring 5 to 7 mm in thickness and of variable dimension is taken and put into a mixture containing 10% formalin, 10% pyridin, 25% acetone, 25% alcohol and 30% aqua distillata for a period of 5 days at room temperature. The tissue is then thoroughly washed in distilled water for 24 hours. Next, it is transferred to 96% alcohol for 3 days and then thoroughly washed in distilled water for 24 hours. After this, the tissue undergoes the following treatment, which is carried out in a dark bottle:

1. Bath in 1.5% silver nitrate solution for 3 days at 37° C (or 5 days at room temperature).
2. Wash in distilled water for several hours.
3. Reduce in 4% pyrogallal solution with the addition of 5% formalin for 24 hours at room temperature.
4. Wash thoroughly in distilled water.
5. Transfer to 80% alcohol for 24 hours.
6. Then to 95% alcohol for 3 days.
7. Absolute alcohol for 2 days.
8. Xylol, xylol-paraffin, paraffin.

The sections should be cut from various strata of the tissue, in order to ensure obtaining the best impregnated zone. The thickness of the section must depend upon the degree of impregnation, which varies considerably according to different specimens of the brain. It is my custom to cut to 3 μ , but it is often preferable to cut to 5 μ , as one thereby increases the chances of finding the pallida in a given area.

It is highly advisable to impregnate a syphilitic tissue containing numerous pallidæ, in order to control the staining of the brain tissue at the same time.

When the staining is successful all the various tissues of the brain appear in a color varying from a pale yellow to a yellowish brown, while the pallidæ are pure black. The neuroglia fibres sometimes stain distinctly, but when examined with artificial light they are

found to be brownish but never black. The sections in which the fibres appear pure black in color are seldom suitable for showing the pallidæ and the staining may thus be considered a failure, although I have on rare occasions succeeded in utilizing such preparations. It is always my rule in commencing my search for the pallida to start from the palest area and proceed gradually towards the edge, which usually takes on a deeper impregnation.

The above method is also applicable to cases of tabes dorsalis. It is best to cut the spinal cord 2 cm long and to make longitudinal sections.

Spirochæta pallida has thus been demonstrated in the brains in general paralysis and also in the spinal cord in tabes dorsalis. The percentage of positive findings is about 25 per cent. in the former, but the organism was found only once in twelve cases of tabes. Although it may seem strange that it was not found in all the cases, we must take into consideration that the search made in my series was rather incomplete, as in the majority of cases only one region of the cortex was studied and of this only a few sections. There can be no doubt but that a complete examination of each case would reveal the *Spirochæta pallida* in all of them. Owing to the tediousness of the technical labor I have been unable to make a more exhaustive search, but I hope that other workers will, by their investigations, be able to supplement the present study.

The pathogenesis of the characteristic lesions of general paralysis and tabes dorsalis has never been understood before, but now that the organisms have been found in the very seat of the pathological processes it may be assumed that these lesions are the result of the immediate presence of the pallidæ. In general character the changes produced are merely those of a chronic parenchymatous encephalitis brought about by the invading spirochætæ.

Thus the discovery of *Spirochæta pallida* in cases of so-called general paralysis leads us to hope that the way may be prepared for the solution of many therapeutic problems which have puzzled science. Thanks to the discovery of salvarsan and neosalvarsan, one of the most powerful weapons by which the commonly known forms of disease produced by this microörganism is being com-

bated, this malefactor is being more and more brought under control. Is it then too much to hope that the same master-mind of Ehrlich will again come to our help in supplying a remedy for this particular kind of syphilitic affection, the baneful effects of which upon human society are too well known to need mentioning and opposed to which we still stand in utter helplessness?

CULTURAL RESULTS IN HODGKIN'S DISEASE.*

BY C. H. BUNTING, M.D., AND J. L. YATES, M.D.

(From the Pathological Laboratory of the University of Wisconsin, Madison.)

The publication at this time of the result of our endeavors to cultivate an organism from the tissues of patients with Hodgkin's disease is occasioned by the publication by Negri and Mieremet¹ of the successful cultivation from two cases of Hodgkin's disease of a diphtheroid organism which they designate as the *Corynebacterium granulomatis maligni*.

While Hodgkin's disease, or malignant granuloma of the lymphatic apparatus, has of late years come to be generally regarded as of infectious nature, the etiological agent has remained undetermined. The work in this country of Reed² and of Longcope³ showed definitely that the condition was independent of tuberculosis, and that the theory supported by Sternberg⁴ that it was a manifestation of the activity of the tubercle bacillus could not be maintained.

In 1900 Fraenkel and Much⁵ reported that by treating Hodgkin's nodes with a strong alkaline solution of sodium hypochlorid (antiformin) they had found in the sediment, in twelve out of thirteen cases, certain granular, Gram-staining, but non-acid-fast bacilli, which they considered to be non-acid-fast tubercle bacilli, "possibly identical with the ordinary tubercle bacillus," but "more probably a special form of the tubercle virus," or at least "belonging to a related group of organisms."

* This work has been aided by a grant from The Rockefeller Institute for Medical Research. Paper read before the Association of American Physicians, May 7, 1913. Submitted for publication, June 5, 1913.

1. Negri and Mieremet: Centralbl. f. Bakteriol., 1913, lxviii, 292.
2. Reed: Johns Hopkins Hosp. Reps., 1902, x, 133.
3. Longcope: Bull. Ayer Clin. Lab., 1903, No. 1.
4. Sternberg: Ztschr. f. Heilk., 1898, xix, 21.
5. Fraenkel and Much: Ztschr. f. Hyg., 1910, lxvii, 159.

In the present year Negri and Mieremet¹ have reported the successful cultivation on Bordet's medium (blood-glycerin-potato-agar), from two cases of Hodgkin's disease, of an organism which falls within the diphtheria group and further agrees in morphology with the forms described by Fraenkel and Much. The organism is a non-acid-fast, Gram-staining bacillus, growing luxuriantly at body temperature, and is a facultative anaerobe. Perhaps its most striking feature is its pleomorphism. The organism showed such variability in form that it was only after isolation of a single organism and cultivation of it that the authors were satisfied that they were dealing with a pure culture. In various media the following forms were found: Plump, short rods, some so short as to resemble coccobacilli, these latter forms predominating or being exclusively found in old cultures; small thin bacilli with polar-staining; comma-shaped bacilli; granular rods of variable size, often of considerable length; branching forms; and on all media, club-shaped involution forms and large spherical forms. This organism is resistant to antiformin and the authors consider it identical with the organism described by Fraenkel and Much.

In a study of Hodgkin's disease, extending over the past five years, we have made various attempts to secure cultures from material removed at autopsy and at operation. The first attempts were made with ordinary media, and were unsuccessful. In Case 9 of our reported series,⁶ cultures were made at operation, in October, 1910, by planting portions of lymph-nodes in bouillon, but apparently in this case there was a complicating infection, as only a streptococcus growth was obtained. In Case 5 of the series, attempts were made to secure cultures from the enlarged mesenteric nodes at autopsy, in January, 1912, but post mortem invading organisms completely overgrew the media.

After these failures, it was felt that special media must be used, and as at this time we felt from certain observations that the organism was probably one of the higher forms of bacteria, if not a fungus, media appropriate to the growth of these organisms were selected. Dorset's egg-medium and glycerin-phosphate-agar were first tried. The first opportunity for their use came in an operation

by one of us (J. L. Yates) for the removal, on Feb. 13, 1912, from a woman patient, of a large group of cervical lymph-nodes.

The essential points in her history are as follows:

Case 1.—Female, white, age 33. When first seen in October, 1911, the patient showed in the right side of her neck a group of enlarged, discrete, lymph-nodes extending from the mid-clavicular region upward in the posterior triangle practically to the attachment of the sternocleido-mastoid. This group of nodes had shown progressive enlargement during the year previous. Except for one small node below the clavicle on the right side and a rather large gland in the right axilla, no further enlargement of lymph-nodes was noted at this time. The spleen was not palpable. The tonsils were enlarged. October 10, a von Pirquet test and a Wassermann reaction were both negative. October 23 the large axillary node was removed for diagnosis. In section it showed hyperplasia of lymphoid and of endothelial elements with giant cells of the endothelial type and of the megalokaryocyte type, disappearance of the architecture of the node, and a beginning diffuse sclerosis and marked eosinophilic infiltration. December 11, tonsillectomy was done, and Feb. 13, 1912, the large group of lymph-nodes in the right side of the neck with the intervening sclerotic tissue, was removed en masse.

After removal the group of nodes was incised, and, with the observation of the strictest aseptic technic, portions of the nodes and of the interglandular tissue were planted on the surface of slants of the egg-medium and of the glycerin-phosphate-agar. The tubes were sealed with paraffin and incubated at 37 C. The tubes were apparently sterile at the end of ten days' incubation, but they were left undisturbed in the incubator, and at the end of three weeks' time a slight growth was noted on one tube. March 12, two of the egg tubes and one tube of glycerin-phosphate-agar showed pure cultures of a diphtheroid organism, with many clubbed-shaped involution forms, with granular and barred rods and evidently branching filaments. The organism was Gram-staining.

Subcultures were made from the original tubes on blood-serum, egg, glycerin, agar, and the ordinary media. The organism proved to be a rather feeble grower under the conditions under which its growth was attempted at that time. On blood-serum in twenty-four hours there were obtained the characteristic shining, opaque, white colonies. On agar the growth was slow, the streak at first being of a grayish, glistening, translucent appearance, becoming more opaque with age. In bouillon there was a scanty growth which gradually settled to the bottom as a somewhat slimy precipitate. No turbidity of the medium occurred. Milk was not coagulated. Its reaction was changed but little if any.

The growth was tested on guinea-pigs, and while the results were unsatisfactory in a sense, it was determined that the animals would survive inoculation with a heavy dosage of a twenty-four-hour culture for ten days at least. Through an accident this culture was lost before thorough testing of it could be done.

Further opportunities for obtaining cultures were not obtained until January, 1913, when, through the kindness of Dr. W. J. Mayo and the cooperation of his staff, the courtesies of the clinic at Rochester were extended to us.

On Jan. 9, 1913, cultures were made from a cervical gland removed for diagnostic purposes from a patient with the following history:

Case 1.—Female, white, aged 34 years. Four years previously she noticed a swelling on the right side of her neck, followed by the appearance of other small tumors. Ten months before date enlarged lymph-nodes appeared on the left side of the neck and in the right axilla. Six weeks previously nodes appeared in the left axilla. Physical examination revealed, in addition to these nodules, a mediastinal mass, a six months' pregnancy, over which were felt discrete firm nodules which slipped under the examining hand. Nose and throat examination revealed a hypertrophic rhinitis, causing some nasal obstruction and also enlarged tonsils. Red blood-cells, 4,480,000; leukocytes, 12,400.

Pathology of Node.—The test node shows microscopically loss of architecture, with lymphoid and endothelial hyperplasia, the presence of many endothelial giant cells, a beginning fine sclerosis and eosinophilic infiltration. There is slight invasion of the capsule.

Pieces of the gland were planted on egg-medium and on Loeffler's blood-serum. The tubes were sealed and incubated. On January 17 one egg-tube showed the characteristic clubbed and granular diphtheroid forms seen in the first culture; but associated with a growth of *B. subtilis*. One serum-tube showed a pure culture of the diphtheroid organism, and one serum-tube showed the presence of a white staphylococcus. Three other tubes were sterile.

Subcultures of the diphtheroid organism were made from the serum-tube onto other media, particularly egg-medium, glycerin-phosphate-agar and plain agar. The growth proved to be luxuriant on these. The top of the streaks had a grayish glistening appearance, while the lower part of the streaks was at first of an opaque white appearance, gradually assuming a slight yellowish color, deepening in very old cultures, on glycerin agar, to a brownish. Smears from the older cultures showed such a pleomorphism, and there were so many coccoid forms it was at first thought we were dealing with a mixed culture of bacillus and coccus. It was only after growth of many generations on egg-medium, on which the culture is of a pure bacillary type during the first twenty-four hours, and after shaking the culture with sand and plating from a further dilution, which gave colonies of a uniform and characteristic type, that we became assured we were dealing not with a symbiosis of coccus and bacillus, but with a pleomorphic organism.

A third successful cultivation was made Feb. 15, 1913, from a cervical lymph-node from the following case from the Mayo Clinic:

Case 3.—Married female, white, aged 38, with marked enlargement of the cervical nodes and rather general glandular enlargement. The illness began with involvement of the cervical glands two years previous to date.

The node removed for diagnosis showed extreme sclerosis, but with scattered alveoli-like areas in which the characteristic elements of the Hodgkin's lymph-node, the endothelial cells, giant cells and lymphocytes were preserved. Eosinophilic infiltration was well marked.

After one week's cultivation there were secured from two tubes, one of Loeffler's serum and the other of egg-medium, a pure culture of a diphtheroid, pleomorphic organism apparently identical with that secured from the previous case, showing the same cultural characteristics. It was put through the same procedure to determine the purity of the culture and gave the same result, that is, after shaking with sand and diluting and plating, all the colonies on the plates were of a uniform character.

In the interval between the last two cases, attempts were made to secure cultures from two other cases, but they resulted less successfully. The first of these attempts was at a post-mortem, Jan. 15, 1913, on the following case:

Case 4.—Female, white, aged 50 years (patient of Dr. Reeves of Albany, Wis.). The glandular swelling had first appeared in the left cervical region two years before. The cervical group of lymph-nodes had been removed about six months previous to the patient's death. At the post-mortem examination there was found rather general glandular involvement, but especial enlargement of the mesenteric and retroperitoneal nodes and of the lymphoid elements of the spleen. There was also a chylous ascites, well marked anemia and emaciation. Old pleural scars were noted at the pulmonary apices and one calcified focus in a bronchial lymph-node. No other signs of tuberculosis were seen.

Microscopically, the abdominal lymph-nodes showed a well-marked hyperplastic Hodgkin's disease picture with beginning diffuse sclerosis, while the splenic lesions showed a more advanced sclerotic condition.

On one Loeffler serum-tube, in which was planted a piece removed from the interior of the spleen, there was noted a growth on the tissue itself of typical polar-staining diphtheroid organisms, but for some reason unascertained, they would not "catch on" to the medium, and attempted subcultures from scrapings of the tissue on both the egg-medium and on serum proved negative.

The second case was the following from the Mayo Clinic, Jan. 31, 1913:

Case 5.—Male, white, aged 33. The patient had had an abscess of a tooth six months previously. About three weeks before coming to the hospital patient had noticed a painless swollen node in the left supraclavicular region. This had increased in size during the time of observation. The large node removed was of a uniform medullary appearance on gross section, and microscopically showed lymphoid and endothelial hyperplasia, with the presence of giant cells, beginning diffuse sclerosis and eosinophilic infiltration. The architecture of the node was destroyed.

Unfortunately for the success of cultural investigation, this gland had been handled and incised before the plants on the media were made. Four days after incubation of the tubes was begun, there was found on one of the serum-tubes a growth of the typical diphtheroid organism, but in association with a coarse bacillus of the *B. subtilis* type. As at this time our efforts were being directed to the elimination of the supposed coccoid contamination of our second culture, no attempt was made to separate the diphtheroid organism from this last mixed culture.

While we have made no systematic attempt to find the organism described in histological preparations from the nodes of Hodgkin's disease patients, one case should be recorded as of interest in connection with the cultural results. Portions of the organs from a girl of 6 years, a patient of Dr. Ogden of Milwaukee, who died in a severe vomiting attack, were received in the laboratory. The Peyer's patches of the intestine and the mesenteric lymph-nodes showed the changes of early Hodgkin's disease, which from clinical and post mortem evidence must have been primarily intestinal in origin. On staining the sections by the Gram-Weigert method, the only organisms found, save for the surface intestinal bacteria, were groups of polar- and granular-staining diphtheroid organisms lying deep within the enlarged Peyer's patches.

SUMMARY OF CULTURES.

To summarize our cultural results: In three cases of Hodgkin's disease we have secured a pure culture of a pleomorphic diphtheroid organism. In two other cultural attempts the organism was recognized, but was not secured in pure culture, and in a sixth case a similar organism, morphologically, was stained in the lesions of a primary intestinal Hodgkin's case.

The detailed biological reactions of this diphtheroid organism have as yet not been completely worked out by us. The strain recovered from the second case, however, has been found to grow readily at 37 C. on the media used to secure the cultures and on ordinary agar-agar. On glycerin-phosphate-agar the growth is almost as luxuriant under strict anaerobic as under aerobic conditions. For luxuriant growth, marked moisture of the medium seems necessary. On a relatively dry medium, growth is slow, and the organisms are found to develop as the long forms, granular, banded, and with many club-shaped involution forms. Branching forms are also noted. These forms are especially well developed on the egg-medium, where they also seem to have a tendency to cohere, so that in stained smears one gets many small groups of organisms radially arranged, with clubbed peripheral elements, somewhat suggestive of a minute actinomyces colony, as seen in section. On moist serum tubes with luxuriant growth, the organisms are short and plump,

with polar-staining. Many of these forms are coccoid. We have noted also, as emphasized by Negri and Mieremet, that in all old cultures coccoid forms predominate, and also that large spherical involution forms are present. A colony or a streak, which at twenty-four hours shows entirely the bacillary form, will twenty-four or forty-eight hours later show an apparent outnumbering of the bacilli by the coccoid elements.

The organism stains by the Gram method, though the short plump forms hold the dye less strongly than the longer bacillary forms. It is not acid-fast. No spore formation has been noted.

The growth of the organism is at first glistening and grayish, but becomes more opaque and of a white color. Apparently, depending somewhat on the reaction of the media, there may be in some early cultures a slight greenish-yellow tint produced. Old cultures on glycerin-phosphate-agar become brownish, and the medium itself darkens.

Gelatin is not liquefied. There is no early change in reaction in litmus milk. Bouillon is not clouded by the growth. Flecks appear along the side of the tube, and a slimy deposit gradually accumulates at the bottom.

Plate cultures show a rounded colony with quite regular edge, a fine stippling of the growth and a central dark spot. The colonies are of a glistening gray color at the end of twenty-four hours, becoming gradually of an opaque white color.

Altogether, our studies thus far seem to indicate that we are dealing with the same organism described by Negri and Mieremet, and while our results taken with theirs cannot be said to indicate positively that we are dealing with the cause of Hodgkin's disease, yet they are strongly suggestive. The morphological elements obtained by Fraenkel and Much by the antiformin method in twelve out of thirteen cases are so similar to the organism cultivated by us that we find added support in their findings to the importance of the organism in the disease. Ultimate proof can come only from an extended series of cultures, or from animal experimentation. The latter test is now in progress in this laboratory with a variety of animal species. Should the relation of the organism to the disease be established, we would suggest *Corynebacterium Hodgkini* as a more

appropriate name for the species than the trinomial designation suggested by Negri and Mieremet.

Although the utmost efforts were made to prevent carrying in organisms from the skin, when removing nodes for cultural investigation, in almost every case studied, one or more tubes have shown the presence of a white staphylococcus. This, when taken in connection with the polymorphonuclear leukocytosis, which occurs late in the disease, has suggested that possibly a secondary infection plays a part in the development of the disease. This feature also needs further investigation.

ADDENDUM.

After this paper was placed in the hands of the publishers, the opportunity was presented to make cultures in another case of Hodgkin's disease from a patient in the charge of Dr. Frank Billings of Chicago. The essential points in the patient's history are as follows:

Male, white, aged 32. Loss of weight since Jan. 1, 1913. During a period of six weeks before date of examination (April 23, 1913) gradual enlargement of the nodes in the neck and groin and to some extent in the axillae was noted. On examination there was found moderate enlargement of the tonsils, cervical, axillary and inguinal nodes and a firm palpable spleen.

April 28, blood-count was, R. B. C., 4,150,000; W. B. C., 4,150. Hgb., 70 per cent. (Dare). April 28, a node from the left side of the neck was removed by Dr. Dean Lewis. This showed on section endothelial proliferation with endothelial giant cells, some fine diffuse sclerosis and but little eosinophilic infiltration.

May 7, Dr. F. B. Moorehead extracted right upper first molar and second bicuspid teeth, finding a large amount of granulation tissue around the roots, and along the lingual root of the molar an abscess cavity about 1 cm. in length containing pus of foul odor. Unfortunately, no cultures were made.

May 29, with the observation of the strictest precautions to prevent contamination, Dr. Lewis removed a node from the left cervical region, and one from the right inguinal region.

In the laboratory of Dr. E. C. Rosenow, and with his assistance, which is gratefully acknowledged, pieces of these nodes were planted on Loeffler's serum, egg-medium, blood-agar and serum-glucose-agar. Other portions of the nodes were ground in a sterile mortar and the emulsion planted on tubes of the media mentioned.

From these plants growth of the diphtheroid organism was obtained in pure culture on Loeffler's serum from both the cervical and the inguinal nodes where solid pieces of the node were used. The organism was also obtained from the cervical node, where the emulsion was planted, but with a contaminating pigment-producing air organism.

Dr. Rosenow reports positive results from the cervical node on blood-agar slants, aerobic and anaerobic, and from the depths of one tube of serum-glucose-agar. From the inguinal node two colonies developed in the depths of the serum-glucose-agar tube.

THE HISTOGENESIS OF BLOOD PLATELETS.*

By WADE H. BROWN, M.D.

(From the Pathological Laboratory of the University of North Carolina,
Chapel Hill.)

PLATE 9.

The problem of the histogenesis of blood platelets has been attacked from many points of view, but none of the theories advanced seems to be wholly satisfactory. Among the causes which have contributed to this confusion is the failure to recognize the unity in character of all blood platelets, and, until recently, the inability to reproduce the structural and tinctorial characteristics of blood platelets in section as they appear in smears stained by any of the stains of the Romanowsky type. The methods of staining blood platelets in section devised by Wright¹ and by Schridde² have obviated the technical difficulties of the problem and have served to emphasize the idea of the unity of blood platelets.

By means of a special method of technique Wright succeeded in staining blood platelets in section as characteristically as in smears, and he was further able to demonstrate the existence of a parent protoplasm with identical structure and staining characteristics in the megakaryocytes of the blood-forming organs. This theory of the origin of blood platelets from the megakaryocyte has been confirmed by Bunting,³ Ogata,⁴ and others. It is especially convincing on account of the ease and clearness with which the essential facts can be demonstrated.

In tracing the development of platelets in embryonic and adult

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¹ Wright, J. H., *Boston Med. and Surg. Jour.*, 1906, cliv, 643; *Jour. Morphol.*, 1910, xxi, 263.

² Schridde, H., *Anat. Hefte, 1te Abt.*, 1907, xxxiii, 1.

³ Bunting, C. H., *Jour. Exper. Med.*, 1909, xi, 541.

⁴ Ogata, *Beitr. z. path. Anat. u. z. allg. Path.*, 1912, lii, 192.

tissues, however, Wright refers to only two types of cells in mammals that possess a blue staining protoplasm with purple granules analogous to those of the platelet; namely, the megakaryocyte and their forerunners in embryonic tissues, or, as he calls them, "small megakaryocytes." One is left uncertain, therefore, as to whether other tissue cells possess a protoplasm of like character, or whether this type of protoplasm is restricted to the two cells named. We know from smear preparations that this is not the case with the cells of the circulating blood, as the transitional leucocyte possesses a granular protoplasm quite like that of the platelet, and to a less degree azure granulations are occasionally observed in other mononuclear leucocytes. We might infer, therefore, that such narrow limitations as Wright's work implies could not be placed upon the distribution of protoplasm with the characteristic azure granulation of platelets, and the question naturally arises as to whether cells with such protoplasm might not participate, to some degree, in the formation of blood platelets. It was with these points in mind that an investigation of the distribution of platelet-like protoplasm and the participation of this protoplasm in the formation of blood platelets was undertaken.

The material used in the investigation was taken largely from the rabbit and the guinea pig, although some human tissue was used. The Schridde and the Wright methods of staining were used as the basis of the technique. Both methods, especially that of Wright, permit of considerable elasticity in their use. A brief note of some of the points that I have found of advantage in the application and modification of this technique is of importance.

TECHNIQUE.

Tissues.—Tissues for platelets should be obtained as fresh as possible, but good granular stains of megakaryocytes and other cells can be obtained from tissues not fixed for eight to ten hours after the death of the animal.

Fixation.—The saturated solution of bichloride of mercury in 0.9 per cent. salt solution, recommended by Wright, is probably the best fixative for both the Wright and the Schridde stains.

Almost any other fixing fluid will give good results, except for the finer details. Formalin, methyl alcohol, grain alcohol, Orth's fluid, and even Zenker's fluid preserve both cell granules and platelets and permit of clear, differential stains. Of these, methyl alcohol, Orth's fluid, and formalin can be recommended.

Imbedding and Sectioning.—Any method of paraffin imbedding is permissible; there is little or no choice so far as the effect upon the staining is concerned. Sections should be as thin as possible.

Staining.—This is the important feature of the technique, and with both the Wright and the Schridde stains it must be varied according to the fixation of the tissue and the results desired. The proportion of polychrome methylene-blue to eosin now recommended by Wright is 1 to 10.⁵ I have found that it is essential to vary these proportions according to the fixation of the tissue in order to procure the best results, and suggest the following scheme of variations in the composition of the staining mixture with an average preparation of the polychrome methylene-blue, according to Wright, stated in volumes of the eosin solution to one volume of the polychrome methylene-blue: after fixation with bichloride of mercury, 8 to 10 volumes of eosin; after fixation with methyl alcohol, 10 to 12 volumes of eosin; after fixation with formalin or Orth's fluid, 12, 14, or 16 volumes of eosin; after fixation with Zenker's fluid, 7 or 8 volumes of eosin. The mixtures are not stable and should be prepared and diluted with an equal volume of distilled water immediately before use in order to secure constant results. With the Giemsa stain, used by Schridde, similar variations in dilution are advisable. The time of staining varies from ten to thirty minutes, as the proportion of eosin in the mixture is increased. The weaker methylene-blue mixtures with a longer time in a warm oven are preferable. The best results, considering all types of cells, are obtained with tissues fixed in Wright's bichloride of mercury salt solution and stained with Schridde's stain.

Dehydrating, Clearing, and Mounting.—These steps are best carried out with pure acetone, oil of turpentine, and turpentine colophonium, as indicated by Wright.

⁵ Mallory, F. B., and Wright, J. H., *Pathological Technique*, 5th edition, Philadelphia and London, 1911, 373.

The adjustment of the proportions of the components of the staining mixture to the fixation or staining affinity of the tissue is of prime importance. Formalin-fixed tissue is difficult to stain with eosin, while tissue fixed in bichloride of mercury has a strong affinity for this stain, and this principle has been found applicable to the polychrome methylene-blue mixtures. The mixtures of Wright's stain with small amounts of methylene-blue are especially advantageous in demonstrating cell granules, but are apt to stain the cytoplasm of platelets extremely faintly or not at all. A stain that contains sufficient methylene-blue to impart the characteristic blue color to the platelet protoplasm will inevitably overshadow much of the finer granules of other elements, except the megakaryocytes. The staining of these cells is most brilliant with the Wright stain. The superiority of the Giemsa stain with tissues fixed in bichloride of mercury is that all elements are brought out clearly and characteristically.

OBSERVATIONS.

No exhaustive investigation of the occurrence of protoplasm with azure granulations has been attempted, but sufficient work has been done to show that it is not confined to platelets and megakaryocytes nor yet to blood or marrow cells. Such protoplasm has been found in the embryonic trophoblast, the foreign body giant cell with abundant protoplasm, the well preserved tubercle giant cell, the giant cells of sarcomata, and the syncytial masses of chorionic epitheliomata. None of the cells shows a cytoplasm that could be regarded as identical with the cytoplasm of the megakaryocyte, except the syncytial masses of trophoblast and the giant and small cells of the giant-celled osteosarcoma. The other cells mentioned show pale to dark blue cytoplasm and usually dust-like granulations that are easily overshadowed if the cytoplasm be stained too intensely blue. The fragments of such protoplasmic masses are usually abundant in the tissues and strongly resemble platelets. In fact, cell detritus and granular precipitates of many kinds may show the purple granular staining, but the absence of the hyaline blue cytoplasm usually distinguishes such detritus from blood platelets.

It is obvious that such cells and cell detritus are in no way con-

cerned with platelets, and attention is called to them merely to emphasize the fact that the protoplasm is not restricted to cells forming platelets, lest it be assumed that the possession of such protoplasm by cells of the marrow or blood implies a parental relationship to the blood platelet. On the other hand, it should be understood that all megakaryocytes do not necessarily show the same degree of granular staining or, in fact, any granulations at all. The number, size, and staining tone of megakaryocyte granules are extremely variable.

The existence of a group of cells in the circulating blood with platelet-like granulations has been referred to. In the young rabbit these cells are more numerous than in the adult, and a study of the spleen and femur marrow of young rabbits showed that the cells were clearly demonstrable with dilute methylene-blue mixtures of Wright's marrow stain. In order to study these cells with greater advantage, procedures were adopted which destroyed the platelets in the circulating blood and caused proliferation of marrow cells. Injections of alkaline hematin were largely used for this purpose, as its effect upon the various elements of the blood and marrow had recently been worked out, and it gave promise of the type of reaction desired.

In marrow thus stimulated to heightened activity, the endothelial cells covering the surface of the marrow begin to proliferate; they increase enormously in size and become multinucleated, their cytoplasm is vacuolated and stains blue with a rich purple granulation. The granules are irregular; some are extremely fine while others are comparatively coarse. The cell shown in figure 1 is such a cell in an early stage of hyperplasia. These cells continue to increase in size, becoming large multinucleated syncytial masses with pseudopods that burrow into the depths of the marrow. Occasionally one of these pseudopods penetrates the wall of a capillary vessel and segments into small round, oval, or irregular masses with the typical structure and staining of blood platelets. The character and staining of the cytoplasm, the arrangement of granules, and mode of segmentation of the pseudopods of these cells are practically identical with those of the megakaryocyte, except that the processes are usually finer, shorter, and segment more rapidly. The platelets, therefore,

are smaller and more irregular than those formed from the megakaryocyte, but are identical in other respects. Figure 2 shows an endothelial cell mass of this nature, a polykaryocyte, and demonstrates conclusively the impossibility of confusing these structures with the megakaryocytes as well as the fact that they give rise to structures that, from all available criteria, we must regard as blood platelets.

Most of the processes from these hyperplastic endothelial cells are not directly concerned with platelet formation, however, but, as they burrow into the depths of the marrow, segment off from the parent mass and form a new generation of round, oval, or irregular cells. Some of these are distinct myeloblasts with scant dark blue staining cytoplasm and dust-like purple granules that are distinguished with difficulty, while others are larger and possess a more abundant dark blue to pale blue staining cytoplasm with coarse or fine purple granules. These cells are neither ordinary myeloblasts nor myelocytes. In rabbit marrow they are readily distinguished from myelocytes as there are no myelocytes that possess azure granulations. To bring out this distinction, marrow fixed with bichloride of mercury and stained with the Giemsa stain is of especial advantage. In rabbits that have received several large doses of alkaline hematin intravenously the platelets are reduced to a very low level and the megakaryocytes in the marrow of the femur show pyknotic nuclei and a hyaline cytoplasm with few or no granules. The megakaryocytes are necrotic and functionless. The marrow now shows a rapid and peculiar hyperplasia, the cells formed being mainly the large mononuclear or transitional cells with pale blue staining cytoplasm and fine purple granules. Various orders of cells that pass by slight gradations from the ordinary myeloblast to the megakaryocyte can be identified. Some are like the transitional leucocyte of the blood, and the entire group is comparable to the embryonic forerunners of the megakaryocyte.

The cytoplasm of the cells is frequently ragged or shows distinct pseudopods, some of which extend into adjacent capillaries, and the cells themselves enter the blood stream in large numbers. Within the vessels of the marrow most of the cells preserve a comparatively clear cut outline, while only a few show fine, irregular streamers of

platelet-like protoplasm segmenting into platelets. In the splenic sinuses, where they collect in large numbers, the segmentation into platelets is more abundant. Figure 3 shows some of the more common appearances presented by the cells in the splenic sinuses.

Examination of the blood shows an increased number of large mononuclear and transitional leucocytes and an exceptionally large number of these cells with azure granulations. The morphology of the cells in smears is much the same as in sections from the marrow and spleen.

As far as this group of cells is concerned, poisoning with saponin produces the same type of reaction. The myeloid metaplasia occurring in the spleen shows clearly the hyperplasia of premegakaryocytes and the formation of platelets directly from them. This mode of platelet formation has been observed once in the spleen of an old rabbit with a spontaneously developed aplastic condition of the marrow. The case shows that such a mode of platelet formation may exist under pathological conditions that are not of experimental origin.

The morphological evidence from marrow, spleen, and blood points clearly to the formation of blood platelets from the group of marrow and blood cells described above under conditions of excessive demand and incapacity of the megakaryocytes. This evidence was further strengthened by parallel studies of blood and marrow which showed that, after destruction of platelets and megakaryocytes, regeneration of platelets to the normal might occur while there was yet but slight evidence of recovery of the megakaryocytes.

Numerical changes in the elements of the circulating blood furnish still further evidence of a relationship between the mononuclear transitional cells with azure granulations and the blood platelets. The increase in absolute numbers of the cells and the increase in platelets after intravenous injection of hematin are practically parallel. As the normal platelet count is reestablished the cells gradually sink to their normal level. It is recognized that such changes as these may be only a matter of coincidence, and as the majority of blood platelets are probably formed in the marrow or spleen, under all conditions, evidence of this character is only suggestive. Still, there is at least one condition, Hodgkin's disease, in

which the high platelet and transitional leucocyte counts occur together as the most characteristic features of the blood picture.⁶

DISCUSSION.

My investigations have confirmed much of the work of Wright, but they indicate that some modification or extension of his theory of the origin of blood platelets is necessary. The evidence of formation of blood platelets from the megakaryocyte is conclusive. Except for the origin of platelets in the early embryo from a forerunner of the megakaryocyte, Wright recognizes no other source for blood platelets in mammals. Although the megakaryocyte is undoubtedly the principal source of blood platelets in normal adult life, there is sufficient evidence to indicate that the embryonic forerunner, or homologue, of the megakaryocyte, while progressively decreasing with age, never entirely disappears from either the blood-forming organs or the circulating blood, in the latter situation being represented by the transitional leucocyte. It is uncertain to what degree these cells participate in platelet production under normal circumstances. Although I believe that some platelets are normally derived from transitional leucocytes, the evidence to support this belief comes largely from the embryological relations of the cells and their unmistakable participation in platelet formation under pathological conditions. The evidence, therefore, is not conclusive.

Under experimental conditions it has been found possible to destroy the platelets in the circulating blood in large numbers and, at the same time, partially or completely to incapacitate the megakaryocytes. In such instances the regeneration of platelets from giant endothelial cells in the marrow and from premegakaryocytes and transitional cells in the marrow, spleen, and blood is sufficient to disprove the idea of the exclusive origin of blood platelets from the megakaryocyte, unless we accept for all of these cells the designation of small megakaryocytes, applied by Wright to the forerunners of the megakaryocyte in the embryo. Under conditions of such excessive demand for platelets as cannot be met by the normal mechanism of platelet production, there is undoubtedly a greater

⁶ Bunting, C. H., *Bull. Johns Hopkins Hosp.*, 1911, xxii, 369.

or less reversion to an embryonic mode of formation in which cells of a less highly specialized variety assume the rôle of platelet production.

SUMMARY.

1. Under normal circumstances blood platelets are largely derived from the megakaryocyte of the blood-forming organs.

2. The transitional leucocyte, representing a persistent form of the embryonic premegakaryocyte, is a circulating homologue of the megakaryocyte and probably plays some part in normal platelet formation.

3. Under conditions of excessive demand for platelet production, there may be a greater or less reversion to an embryonic mode of platelet formation in which less highly specialized cells than the megakaryocyte participate in platelet production.

4. In addition to the megakaryocyte, the cells that have been observed to take part in platelet formation are hyperplastic endothelial cells in the marrow, and mononuclear and transitional cells (premegakaryocytes) in the marrow, spleen, and blood.

EXPLANATION OF PLATE 9.

The water color drawings were all made with the aid of a camera lucida, and with a Leitz No. 4 ocular and 2 mm. objective. All the figures are from formalin-fixed tissue stained by Wright's method, except the central cell in figure 3, which is from tissue fixed in bichloride of mercury and stained with the Giemsa stain. The composition of the Wright stain used in figures 1 and 2 was methylene-blue 1 part, eosin 12 parts; in figure 3, methylene-blue 1 part, eosin 14 parts.

FIG. 1. An endothelial cell from the surface of the femur marrow of the rabbit, illustrating an early stage of hyperplasia.

FIG. 2. A cell of the same type and situation as that in figure 1, showing marked hyperplasia. A stubby mass of protoplasm in the center is projecting into a capillary and breaking up into blood platelets. The more prolonged mass to the right shows no segmentation, while the process to the left is burrowing through the connective tissue covering the marrow.

FIG. 3. Three types of cells that were numerous in the splenic sinuses of one of the rabbits injected with hematin. The presence of various forms of pseudopods and segmentation into platelets is shown. The platelet count in this animal rose to normal, while the megakaryocytes had regenerated to a very slight degree.

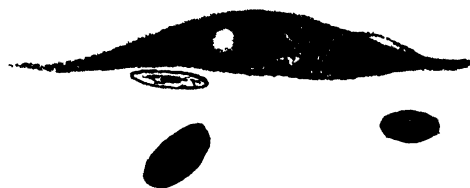


FIG.1



FIG.2



FIG.3

(Brown: Histogenesis of Blood Platelets.)

CONTRIBUTION TO THE CULTIVATION OF THE PARASITE OF RABIES.*

By HIDEYO NOGUCHI, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research,
New York.)

PLATE 19.

Galtier¹ and Pasteur² demonstrated the infectiousness of rabies. Negri³ next described the characteristic inclusions in the ganglion cells, and Babes,⁴ J. Koch,⁵ Volpino,⁶ Proescher,⁷ and others found minute granular or pleomorphic particles in the central nervous system of animals infected with rabies. Remlinger,⁸ Poor and Steinhardt,⁹ Bertarelli and Volpino¹⁰ demonstrated the filterability of the rabic virus; but the nature of the virus itself had remained unknown.

In the early part of 1912 I undertook to cultivate the rabic virus and I have now obtained certain results regarding which a brief report is appended. About fifty series of cultivations were made with the brain or medulla removed aseptically from rabbits, guinea pigs, and dogs infected with "street" virus, "passage" virus, or "fixed" virus. Usually the animals were etherized just before spontaneous death occurred.

The method that has yielded the result to be reported is similar to that employed successfully for the cultivation of the spirochætæ

* Received for publication, August 1, 1913.

¹ Galtier, *Compt. rend. Acad. d. sc.*, 1879, lxxxix, 444.

² Pasteur, L., Chamberland, and Roux, *Compt. rend. Acad. d. sc.*, 1881, xcii, 159.

³ Negri, A., *Ztschr. f. Hyg.*, 1903, xliii, 507; xliv, 520; 1909, lxiii, 421.

⁴ Babes, V., *Traité de la rage*, Paris, 1912; *Ztschr. f. Hyg.*, 1907, lvi, 435.

⁵ Koch, J., *Ztschr. f. Hyg.*, 1910, lxvi, 443.

⁶ Volpino, G., *Arch. per le sc. med.*, 1904, xxviii, 153.

⁷ Proescher, F., *Berl. klin. Wchnschr.*, 1913, l, 633.

⁸ Remlinger, M. P., *Ann. de l'Inst. Pasteur*, 1903, xvii, 834; 1904, xviii, 150.

⁹ Poor, D. W., and Steinhardt, E., *Jour. Infect. Dis.*, 1913, xii, 202.

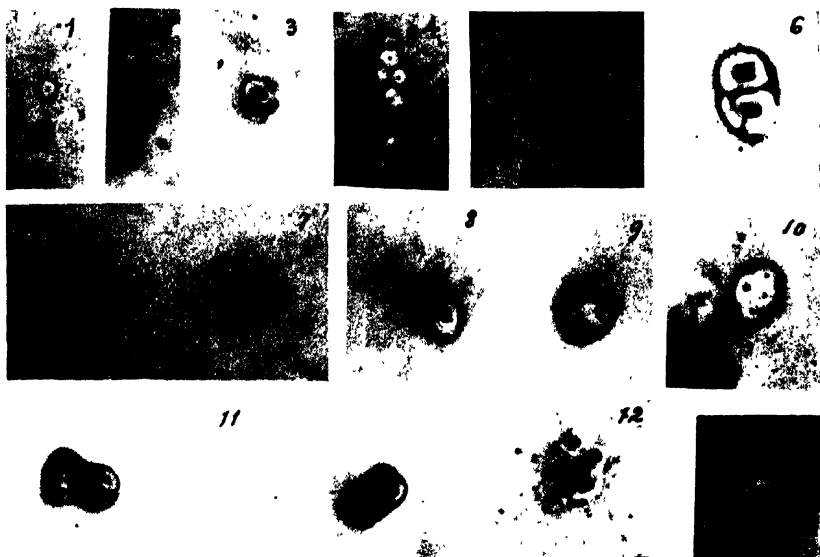
¹⁰ Bertarelli, E., and Volpino, G., *Centralbl. f. Bakteriöl., 1te Abt., Orig.*, 1904, xxxvii, 51; Bertarelli, E., *idem*, 1905, xxxix, 399.

of relapsing fever.¹¹ In the cultures very minute granular and somewhat coarser pleomorphic chromatoid bodies arise which on subsequent transplantation reappear in the new cultures through many generations. The same bodies, so far as appearances are concerned, are obtained from "street," "passage," and "fixed" virus. The smallest of these bodies are just on the limit of visibility with Zeiss apochromatic 2 millimeter lens. On four different occasions I observed in the cultures from "passage" and "fixed" virus nucleated round or oval bodies surrounded with membranes totally different from the minuter granular bodies although arising in the cultures in which the latter occurred. Their appearance was sudden and their duration four to five days, when they diminished coincident with an increase in granular bodies. In one of the four instances the larger bodies appeared in a culture prepared from the brain of a rabbit previously inoculated with a culture of "passage" virus which had given rise to the symptoms of rabies. In two of the instances the larger nucleated bodies arose in cultures prepared from "fixed" virus in which Negri bodies were difficult to demonstrate either in sections or in films.

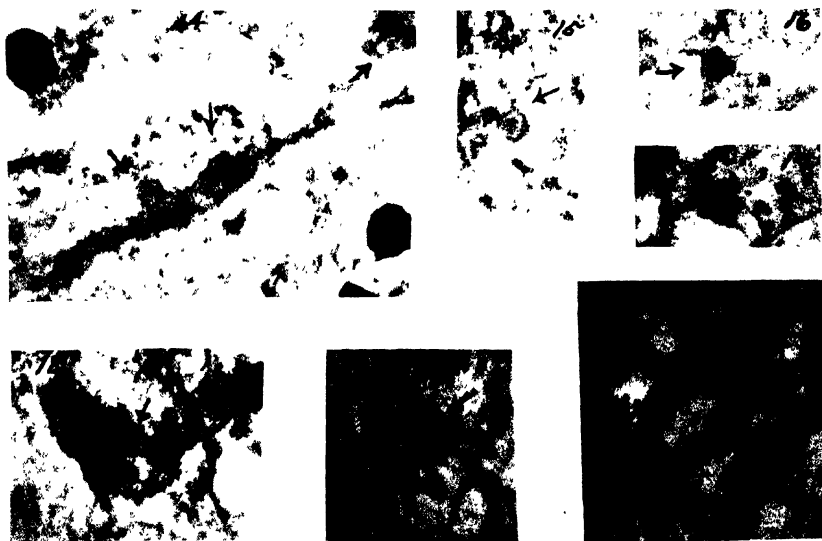
The general morphological characters of the nucleated bodies are shown, magnified 1,100 times in the plate (figures 1 to 13). Along with them are also shown, for comparison, Negri bodies taken from the brain of dogs succumbing to "street" virus (figures 14 to 20). The cultivated nucleated bodies are actively multiplying, by division or budding, and exhibit the appearance not of bacteria but of protozoa. In size they range from about one micron to twelve micra. Under the dark-field microscope, as in the stained preparations, they show definite characters. The center is nuclear and the membrane is distinct and highly refractive. The united individuals in a multiplying mass may be enclosed for a time in a common capsule (?). By inoculating cultures containing the granular, pleomorphic, or nucleated bodies, rabies has been reproduced in dogs, rabbits, and guinea pigs, as shown by the typical symptoms and positive animal inoculations; while the film preparations from the brain of the animals contained always the granular and sometimes the nucleated bodies in large numbers.

¹¹ Noguchi, H., *Jour. Exper. Med.*, 1912, xvi, 199.

A. Nucleated bodies in culture (Giemsa stain).



B. Negri bodies in the brain films (Giemsa stain).



A fuller report on this subject is reserved for a later publication.

EXPLANATION OF PLATE 19.

All microphotographs were taken from the film preparation stained with Giemsa solution. Magnification uniformly $\times 1,100$.

FIGS. 1 to 13. These represent the nucleated bodies of different stages of development in a culture (second and fourth generations, five days old) prepared from the brain of a rabbit experimentally infected with "fixed" virus. In the original material there were no such forms to be seen either in the films or in sections.

FIGS. 14 to 20. These show Negri bodies found in the films prepared from the brain of dogs dying of natural infection with "street" virus. The bodies possess central nuclear substances and distinct cell outlines (membranes). They are usually distributed singly.

THE RENAL COMPLICATIONS OF HEMATIN INTOXICATION AND THEIR RELATION TO MALARIA.*

BY WADE H. BROWN, M.D.

(From the Pathological Laboratory of the University of North Carolina, Chapel Hill.)

Descriptions of the paroxysm¹ and of the changes in the blood² produced by the intravenous injection of alkaline hematin and their relation to human malaria have been presented in previous articles, and it is the object of this paper to add a third group of conditions which may properly be described as the renal complications of hematin intoxication.

The description of these renal complications is based on a study of two classes of material: First, the urine and kidneys of a large series of rabbits, in which the production of renal lesions and disturbances of function were not the objects of the experiments, but were merely incidental; and second, twenty rabbits, in which the production of renal complications was made the prime object of the experiments.

The hematin and hematin solutions used in these experiments were the same as in former experiments. The animals were fed, for the most part, on a variety of green foods, with hay and ground grains, and allowed to take water at liberty. The food in a given experiment was kept constant as to quality throughout the experiment, and the amount of water consumed during twenty-four hours was determined by supplying measured amounts in vessels so arranged that none could be wasted. The water remaining in the vessel at the end of twenty-four hours was measured and corrections for evaporation were made from control vessels. In only a

* Aided by a grant from The Rockefeller Institute for Medical Research. Submitted for publication April 21, 1913.

1. Brown, Wade H.: Jour. Exper. Med., 1912, xv, 579.

2. Brown, Wade H.: Jour. Exper. Med., 1913, xviii.

few experiments were definite amounts of water given by stomach tube. The urine collected from metabolism cages was used for routine study, but the qualitative findings were confirmed by bladder urine in many instances. Observations were made on the normal urine for one or two days before the injection of hematin. In this connection it should be noted that the daily amount and character of rabbit's urine is so variable that but little importance can be attached to slight variations in the amount of urine, the urine-water ratio or the character of the urine. The results of the hematin experiments were controlled by a series of animals that received an equivalent volume of alkaline salt solution under the same conditions as those receiving hematin.

The extreme irregularity in the results of these experiments has shown conclusively that no given dose or combination of doses of hematin can be expected to produce a given effect on the kidneys of the rabbit, and the results reported are to be interpreted as the most probable effect from a given degree of hematin intoxication.

THE URINE.

Amount.—A single dose of 10 mg. of hematin per kilo of body weight, or less, will produce no alteration in the daily amount of urine. If such doses are repeated from day to day, however, slight increases in the output of urine and in the ratio of the urine to the water consumed are gradually developed. The increased volume of urine is also present where slightly larger doses of hematin are administered, but as the dose approaches 20 mg. per kilo, there is a distinct decrease in both the daily amount of urine and the ratio of the urine to the water consumed. In exceptional instances there may be a complete suppression of urine for twenty-four hours or longer. On discontinuing the hematin, this phase of decreased output of urine gives place to increased urinary secretion and to an increase in the urine-water ratio, both of which exceed the normal within two to three days. This change is more enduring—in one animal it persisted for twenty-one days, when observations were discontinued.

Color.—The urine in hematin intoxication is highly colored, varying from a brownish-yellow to a dark reddish-brown and occa-

sionally shows a greenish fluorescence. A smoky-red urine is seen in some instances.

Acidity and Specific Gravity.—The reaction and the specific gravity of the urine show no constant alterations. Both of these features vary widely in the rabbit under normal circumstances, and the variations after injections of hematin are therefore difficult to relate to any action of hematin.

Albumin and Casts.—A trace of albumin and a few hyalin or granular casts may appear in the urine after even a single injection of 10 mg. of hematin per kilo of body weight. The daily repetition of this dose of hematin seldom fails to produce an albuminuria with casts. As much as 20 mg. of hematin per kilo of body weight will produce a marked albuminuria with an abundance of hyalin, granular and epithelial casts, or even blood casts. Neither the amount of albumin nor the number or variety of casts, however, can be predicted from the amount of hematin injected.

Blood and Hemoglobin.—The most interesting phase of disturbed renal function, referable to hematin poisoning, is the occurrence of hematuria and hemoglobinuria. Severe intoxication with hematin frequently results in hematuria of a variable degree. The amount of blood may be so slight as not to be suspected from the appearance of the urine, or so great as to give the urine a distinct red or smoky color. The condition is occasionally produced by a single large dose of hematin (20 to 25 mg. per kilo.); it occurs with greater frequency from the maintenance of a given concentration of hematin in the circulation for twelve to twenty-four hours. This can be done best by injecting three or four doses of 10 to 15 mg. of hematin per kilo within such a period of time. The test on the vitality of the animal is a severe one, and the size of the dose and frequency of repetition must be judged by the condition of the animal; even then many fatalities will result before the desired effect is produced.

Free hemoglobin in the urine, not associated with hematuria, has been observed in a very few instances. Hemoglobin was demonstrated by the guaiacum test in voided urine and bladder urine in five animals out of fifty examined. In two of these cases the guaiacum test was confirmed by the spectroscope. Three other animals

gave a positive guaiacum test from voided urine which was not confirmed by bladder urine. Of these eight animals, all except three showed, post mortem, either hemorrhagic lesions of the kidneys, extensive hemorrhage into the peritoneal cavity, or both, which might have accounted for the presence of hemoglobin in the urine. Of the three undoubted instances of pure hemoglobinuria, one occurred in the course of a chronic intoxication, and the other two from the repeated injection of 20 to 25 mg. of hematin twice daily; one animal developed hemoglobinuria after the fourth injection, and the other after the seventh. All efforts to devise a method of administering the hematin that would produce this condition in a greater percentage of cases proved futile. Most of the attempts resulted in the production of hematuria. It is evident, therefore, that there are other factors than the hemoglobinuria that are essential to the production of a high concentration of hematin in the circulation.

THE KIDNEYS.

Mild hematin intoxication, whether acute or chronic, produces no characteristic lesions in the kidneys. There is a slight increase in the size of the organs and a slight brown pigmentation. The epithelium of the convoluted tubules and of the ascending loops of Henle usually shows parenchymatous degeneration. In the chronic cases a few hyalin casts in the tubules and foci of round-cell infiltration in the boundary zone and in the cortex also furnish evidence of injury.

The injection of large doses of hematin, however, may produce very profound and characteristic lesions. In the acute stages the kidneys are much enlarged and are uniformly dark-brown or purplish-red in color, or are diffusely mottled with small opaque yellow areas of necrosis and areas of hemorrhage. The kidneys are moist, the cortex is thickened and the straight vessels and glomeruli are intensely congested. The boundary zone usually shows the most pronounced congestion and irregular streaks of hemorrhage may extend from this zone into both the cortex and medulla. In rare instances, small infarcts are present. The most characteristic alterations are found in the glomeruli. The glomeruli are enlarged and

their vessels are enormously dilated and congested. In some instances, many of the glomerular capillaries are occluded by hyalin masses that stain a brassy-red with eosin; such glomeruli are apt to show hemorrhage into the capsular space. On the other hand, the glomerular tuft may completely obliterate the capsular space. The glomerular epithelium is very slightly swollen and a few desquamated cells are present. To a less degree, other vessels of the kidney are dilated and congested, while many of the smaller vessels show hyalin thrombi or emboli. Greenish-brown pigment is found, especially in the glomerular capillaries, both as free granules and masses and within phagocytic cells.

The uriniferous tubules, more particularly the convoluted tubules and the ascending loops of Henle, show marked parenchymatous degeneration with desquamation of the epithelial cells, or even foci of necrosis. In extreme instances almost the entire cortical system may be necrotic. Occasionally, the tubular epithelium will show granules of greenish-brown pigment, much of which, as it reacts for iron only after oxidation with hydrogen peroxid³ and is soluble in dilute alkalis, must be regarded as hematin. This pigmentation is more common in living than in necrotic cells. An abundant albuminous precipitate and many varieties of casts are found in the tubules throughout the kidney. Hemorrhage into the tubules is found in many cases of extreme intoxication. The hemorrhage is usually patchy in its distribution—seldom diffuse—and occurs in the region of those glomeruli that show hemorrhage or occlusion of their vessels. In some of these cases, there is also a diffuse staining of the epithelium with hemoglobin and granules or droplets of hemoglobin are found in the cells, in the lumen of the tubules and in the capsular space. The presence of hemoglobin was observed in three instances in which there was no hemorrhage. The presence of hemoglobin was verified by oxidation with hydrogen peroxid and obtaining an iron reaction, and by the presence of brownish, granular and crystalline deposits in the cells and lumen of the tubules in specimens fixed in a solution of formaldehyd immediately after the death of the animal.⁴

3. Brown, Wade H.: Jour. Exper. Med., 1911, xiii, 477.

4. Browicz: Virchows Arch. f. path. Anat., 1900, clxii, 373.

The interstitial tissues are usually edematous and there may be foci of hemorrhage. Foci of necrosis are usually surrounded and invaded by polymorphonuclear leukocytes.

In two instances anemic infarcts were observed from occlusion of relatively large vessels by masses of hyalin material.

In the early stages of recovery from hematin intoxication and at the time the kidneys are showing an increased urinary secretion, several important changes from the above description are to be noted. The glomerular tufts now decrease to normal size or are shrunken, while the capsular spaces and tubules are dilated and contain desquamated epithelial cells and an abundant albuminous precipitate.

In chronic intoxications, and as recovery from acute intoxications progresses, the kidneys are but slightly enlarged, but still show a brown pigmentation. The surface may present a few scattered areas of hemorrhage and necrosis, as in the acute stage, interspersed with small depressed scars. The glomeruli are irregular, some being enlarged while others are much contracted. The tubular epithelium shows various degrees of degeneration, with a slight desquamation, or even patches of necrosis. Many tubules show regenerating epithelium with numerous mitotic figures. In these areas of regenerating tubular epithelium there is usually a pronounced interstitial infiltration of plasma cells and an increase in the connective tissue.

DISCUSSION.

Clinically and pathologically, slight degrees of hematin intoxication cause only such alterations in urine and kidneys as are commonly observed in many febrile conditions. In more severe intoxications the irregularity and uncertainty of the effect on the kidneys is quite striking; some of these cases again show only the disturbances of a febrile state, while others present the clinical picture of an acute nephritis and a very few a definite hemoglobinuria. Throughout, the one constant feature is the albuminuria with casts and degenerative lesions in the kidneys—all probably the result of a slight toxic injury. The wide range of variety in urine and kidney lesions that may be superimposed on this common basis under

constant experimental conditions suggests strongly the element of chance in the effect of hematin on the kidneys. This chance factor is operative through the blood-vessels. It has been demonstrated by physiological methods that hematin produces a marked dilatation of the splanchnic vessels⁵ and causes injury to the vessel wall. Microscopically, the glomerular vessels in particular show these changes and, in addition, obstruction to circulation in many areas by hyalin thrombi or emboli. This occlusion of vessels seems to be the chance occurrence that determines the major part of the renal complications of hematin intoxication. The foci of tubular necrosis are but minute infarcts; the hemorrhage also is largely determined by vascular occlusion, although the injured and weakened vessel wall predisposes to hemorrhage.

A few kidneys have shown such a uniformity in the character and distribution of the lesions that one might be tempted to regard them as instances of acute nephritis, but in view of the great preponderance of lesions that are undoubtedly due to vascular occlusion and injury I am inclined to regard them all as of like nature.

No light has been thrown on the important subject of hemoglobinuria except that it is possible of production by intravenous injections of hematin in a very small percentage of cases. The hemoglobinemia resulting from hematin intoxication undoubtedly reaches the threshold value of a kidney showing vascular and tubular injury at a point below that of the normal kidney.

It is not possible to correlate closely the renal complications of hematin intoxication with those of human malaria. The disturbances of function and the lesions of the kidneys have been found much more pronounced in hematin intoxications than in comparable grades of malarial infection in man, while the predominance of the glomerular lesions observed in these experiments is not found in malaria. These differences are partly due to the difference in the concentration of hematin in the blood. It is believed, however, that the analogy is sufficiently close to render the facts disclosed by these experiments of value as a basis for a clearer comprehension of the mode of production of the renal complications of human malaria.

5. Brown, Wade H., and Loevenhart, A. S.: *Jour. Exper. Med.*, 1913, xviii. (In press.)

SUMMARY.

1. Mild grades of hematin intoxication produce degenerative lesions in the kidneys and the urine shows a trace of albumin and casts.

2. Severe grades of hematin intoxication result in extensive dilatation, injury and occlusion of the renal vessels by hyalin thrombi or emboli, all of which are most pronounced in the glomerular vessels. Extensive degeneration and necrosis of tubular epithelium, hemorrhages and even anemic infarcts result from these vascular lesions. In such cases, the urine presents the characteristics of an acute nephritis.

3. In rare instances of severe hematin intoxication, hemoglobinuria may occur.

4. During the period of recovery from acute hematin poisoning and in chronic poisoning, the kidneys show both degenerative and proliferative processes. The glomerular tufts shrink and the capsular space and tubules are more widely dilated. The tubular epithelium shows degeneration and active regeneration with abundant mitotic figures. There are foci of round-cell infiltration and of connective tissue increase. There is also a slight diffuse increase in connective tissue. The urine is increased in amount and contains albumin with hyaline and granular casts.

5. The renal complications of hematin intoxication are believed to be due primarily to dilatation, injury and occlusion of renal vessels under the action of hematin.

LA LUÉTINE-RÉACTION.

CUTI-RÉACTION DE LA SYPHILIS.

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A la suite des brillantes recherches de Metchnikoff, Ehrlich, Kitasato, Bordet, Calmette, Wright, Madsen, Flexner, Pfeiffer, Wassermann et leurs élèves, apparut sur la scène scientifique un nouveau phénomène biologique nommé par Richet: anaphylaxie. La première observation de Th. Smith et les recherches subséquentes de Richet, Otto, Besredka, Rosenau et Anderson, von Pirquet, Kraus, Doerr, Friedberger, Lewis, Auer, H. Pfeiffer, et beaucoup d'autres savants ont établi la nature spécifique de ce phénomène. Bien qu'on n'ait pas pu encore donner une explication satisfaisante de l'ensemble du phénomène, la spécificité de la réaction a amené différents chercheurs à l'utiliser pour le diagnostic de certaines maladies. C'est ainsi que pour la tuberculose nous connaissons l'épreuve à la tuberculine de Koch, la cuti-réaction de von Pirquet, l'ophtalmo-réaction de Calmette; pour la morve, l'épreuve de la malléine; pour la fièvre typhoïde, l'ophtalmo-réaction de Chantemesse.

La réaction locale basée sur l'hypersensibilité fut utilisée pour la première fois par von Pirquet. Il semble que dans certaines maladies infectieuses chroniques, telles que la tuberculose et la syphilis, ce phénomène désigné par von Pirquet, sous le nom d'allergie, se développe et peut être décelé par un procédé semblable. Mais avant que nous puissions employer ce réactif, il est nécessaire d'obtenir les principes constituants du micro-organisme infectant sous une forme pure et suffisamment concentrée. Ceci explique que le réactif doit être extrait d'un matériel obtenu de cultures pures du micro-organisme en question.

La cuti-réaction de von Pirquet et les autres réactions basées sur

les principes de l'anaphylaxie ne furent possibles qu'une fois que les organismes infectants furent obtenus en culture pure. En ce qui concerne la syphilis, beaucoup de chercheurs ont entrevu la possibilité d'obtenir la cuti-réaction, mais personne n'avait eu l'occasion de réaliser cette réaction, en raison du manque de cultures pures de *Treponema pallidum*; je fus le premier à l'obtenir en 1911.¹ Il est exact que Neisser, Bruck, Tedeschi, Nobl, Ciuffo, Favre, Nicolas et Gautier, Meirowsky, Wolff-Eisner, Jadassohn, Fontana et d'autres savants ont essayé d'obtenir une réaction cutanée spécifique par l'application d'extrait de tissus syphilitiques humains contenant le *T. pallidum*, mais les résultats furent inconstants et non spécifiques, en raison, sans aucun doute, de l'impureté du matériel employé.

Dès que j'eus obtenu des cultures pures de *Treponema pallidum*, j'entrepris des études sur l'immunité et l'anaphylaxie. Les extraits de pallidum furent préparés d'après plusieurs races et appliqués sur la peau d'animaux préalablement sensibilisés ou de personnes souffrant d'affections syphilitiques.

Je résume sommairement la technique de la préparation de la luétine, technique que le lecteur trouvera en détail dans mes publications précédentes.

La luétine est préparée de la façon suivante : des cultures pures de *Treponema pallidum* sont placées dans un bocal stérilisé avec de nombreuses billes de porcelaine, on secoue le tout pendant plusieurs heures, de manière à moudre le tréponème ; on emploie un mélange de cultures liquides et de cultures solides d'âges variés, mais on enlève avec soin, avant de moudre les cultures, le fragment de tissu qui compose un des éléments essentiels du milieu de culture ; il faut obtenir un extrait aussi débarrassé que possible de toute substance étrangère. L'émulsion qui résulte de ces manœuvres doit être parfaitement liquide ; pour cela, on ajoute une quantité suffisante de cultures liquides. L'émulsion est chauffée à 60° C. pendant trente minutes (autrefois, nous chauffions une heure, mais nous avons constaté qu'un temps aussi long n'était pas nécessaire). Autrefois, comme moyen de contrôle, nous préparions une émulsion de milieux

1. NOGUCHI. — "Serum diagnosis of syphilis," 1912, 3^e édit.

de culture ne contenant pas de pallidum, mais nous avons constaté que ce contrôle était inutile.

La luétine que je prépare contient toujours plus de six races différentes de *Treponema pallidum*. Il est bon, pour augmenter la polyvalence, d'employer autant d'espèces qu'on peut.

La luétine est conservée à la chambre froide. Au moment de l'emploi, on la dilue avec une quantité égale de sérum artificiel stérilisé.

L'injection de luétine est faite *dans le derme* de la peau du bras droit; la quantité injectée est de 0 cm³ 07 pour un adulte, 0,05 pour un enfant; on voit apparaître immédiatement une petite papule blanchâtre qui persiste dix minutes environ.

L'injection de luétine détermine *une réaction négative ou une réaction positive*.

QUAND LA RÉACTION EST NÉGATIVE, on constate au siège de la piqure, après vingt-quatre heures, un léger érythème qui a disparu au bout de quarante-huit heures. Parfois, après les vingt-quatre, on constate une papule nettement surélevée, indurée, entourée d'une zone érythémateuse plus ou moins intense; cette papule régresse peu à peu et a disparu le cinquième jour en ne laissant qu'une légère pigmentation; il s'agit là d'une irritation banale causée par l'injection d'une substance étrangère; il ne faut pas confondre cette irritation banale avec une réaction positive.

La RÉACTION POSITIVE peut se présenter sous trois formes: la *forme papuleuse*, la *forme pustuleuse*, la *forme torpide*.

La *forme papuleuse* se manifeste vingt-quatre à quarante-huit heures après l'injection par une papule rouge, indurée, surélevée, d'une dimension de 7 mm. à 10 mm. de diamètre. Parfois cette papule est entourée d'une zone érythémateuse qui, dans certains cas, revêt un aspect légèrement oedémateux. Cet élément papuleux augmente progressivement pendant les deux ou trois premiers jours, devient rouge foncé ou bleuâtre, puis diminue graduellement et finit par disparaître complètement en sept à dix jours; dans certains cas, la papule persiste pendant deux à trois semaines.

La *forme pustuleuse*, dans son premier stade, revêt la forme d'une papule indurée comme dans le type précédent, puis, au bout de quatre à cinq jours, sur la papule on voit apparaître de petites

vésicules et on remarque un début de ramollissement au centre; bientôt se forme une pustule qui contient un liquide d'abord semi-opaque, puis franchement purulent. Le sujet accuse quelquefois un prurit plus ou moins intense.

La pustule se rompt, soit spontanément, soit en raison du frottement des vêtements; une croûte écailleuse se forme et tombe au bout de quelques jours; l'induration diminue; finalement, il reste parfois une petite cicatrice, toujours une pigmentation qui dure plusieurs mois. Dans certains cas la pustule ne s'ouvre pas, elle présente une petite masse centrale fluctuante qui se résorbe.

La *forme torpide* se caractérise par la lenteur dans l'apparition de l'élément typique; le siège de la piqure se décolore au bout de trois à quatre jours, on pense qu'il n'y a aucune réaction, quand brusquement, parfois deux à trois semaines après l'injection, apparaît un élément presque toujours pustuleux qui évolue comme la forme pustuleuse.

La luétine-réaction ne retient pas sur l'état général; parfois cependant on peut noter une petite élévation de la température qui monte à 38 et une céphalée légère qui ne dure pas.

Je n'insiste pas sur ces faits; je veux me borner dans cet article simplement aux résultats obtenus.

D'une façon résumée, on peut dire que la luétine-réaction est pratiquement absente ou très légère chez les personnes présentant des accidents syphilitiques primaires ou secondaires, tandis qu'elle est toujours positive dans les cas chroniques ou latents. Dans les cas de syphilis héréditaire, la réaction est d'ordinaire positive.

Parmi les cas d'affection syphilitique atteignant le système nerveux sous le type cérébro-spinal, la réaction est inconstante; d'après mon expérience, la moitié environ de ces cas indique une réaction positive.

Dans le tabes, la réaction est également irrégulière.

Parmi les cas de paralysie générale, j'ai trouvé deux types différents, ceux qui réagissent positivement: ceux qui ne donnent aucune réaction. Cette absence de réaction s'observe dans les paralysies générales où le processus syphilitique est en évolution, comme le montre la présence de très nombreux tréponèmes pâles; ceci est

seulement un signe léger d'allergie de la peau et peut être rattaché à une sursaturation de l'économie par les antigènes produits dans le système nerveux central, et on peut comparer cet état à la disparition de l'allergie de la peau dans les cas avancés de tuberculose.

Il est intéressant de noter que, chez un syphilitique, la luétine-réaction peut manquer lorsque la réaction de Wassermann est fortement positive et, par contraste, dans les cas de syphilides tertiaires aussi bien que dans la syphilis héréditaire, les deux réactions peuvent être présentes en même temps.

Chez les syphilitiques soumis à un traitement énergique, la réaction de Wassermann graduellement diminue et finit par disparaître, par contre, la luétine-réaction, qui peut être absente ou très légère au début du traitement, devient plus intense au moment où la réaction de Wassermann disparaît.

De tout ce que nous venons de dire, il ressort que la luétine-réaction possède une signification limitée, mais très importante, non seulement pour le diagnostic, mais aussi pour le pronostic. Considérons par exemple un malade qui a contracté la syphilis et qui a subi un traitement énergique; s'il ne présente aucun symptôme ou aucune réaction de Wassermann, on peut dire ou qu'il est guéri ou qu'il est un syphilitique latent; mais si ce malade réagit à la luétine-réaction, nous pouvons le considérer comme un syphilitique latent, en dépit de l'absence de tous les autres signes.

Si cependant la réaction à la luétine est aussi négative, il est peut-être encore prématuré d'affirmer la guérison, mais on peut dire que cette guérison est très probable.

Chauffard, dans son récent discours au XVII^e Congrès international de Londres, a insisté sur l'importance des moyens pronostiques; je crois que, dans de certaines limites, la luétine-réaction remplit ces desiderata pour une maladie dans laquelle jusqu'ici on n'avait aucun moyen d'affirmer la guérison.

Les observations de Milian et de Gennerich en 1910 nous ont enseigné que la réaction de Wassermann peut devenir positive après les injections de Salvarsan chez certains syphilitiques dans lesquels la réaction était tout d'abord négative ou tout au moins indécise. Ce phénomène particulier a depuis été confirmé par des recherches

subséquentes et il est connu sous le nom de *réactivation* du Wassermann. Pour produire cette réactivation, on injecte d'habitude dans les veines 0 gr. 40 de Salvarsan ou 0 gr. 60 de néo-Salvarsan, et on fait l'épreuve du sang le jour suivant ou quarante-huit heures après, et si la réaction est négative, au bout d'une semaine.

Récemment, Nichols a apporté une intéressante contribution à ce sujet. Chez un certain nombre de syphilitiques qui avaient été traités par le Salvarsan et ne présentaient aucun symptôme clinique, ni aucune réaction de Wassermann depuis un an, il appliqua la luétine-réaction à tous, et à quelques-uns en même temps la *réactivation* de la réaction de Wassermann.

Les résultats peuvent être résumés de la façon suivante :

	RÉACTION de Wassermann ordinaire (négative)	LUÉTINE- RÉACT.
	mois	
1.....	15	+
2.....	17	+
3.....	18	+
4.....	19	+
5.....	22	+

GROUPE I.

Dans le groupe I, cinq cas de syphilis, qui ne présentaient aucun symptôme et qui, à plusieurs reprises, avaient donné une réaction de Wassermann négative pendant une période de quinze à vingt-deux mois, réagissaient positivement à la luétine. On constata, par la suite, que ces cas étaient des cas de syphilis latente, car on vit réapparaître des symptômes cliniques et on assista au retour de la réaction de Wassermann. Dans ces cas, par conséquent, la luétine-réaction avait été capable de déceler la latence de la syphilis.

	RÉACTION de Wassermann ordinaire (négative)	LUÉTINE- RÉACT.	RÉACTIVATION de la réaction de Wassermann
	mois		
1.....	16	—	—
2.....	21	—	—
3.....	24	—	—
4.....	24	—	—

GROUPE II.

Le groupe II comprend quatre cas semblables dans lesquels la luétine-réaction et la *réactivation* du Wassermann donnèrent des résultats négatifs. Nichols estime qu'on peut considérer ces cas comme guéris. Ces derniers malades étant soldats, pourront être observés un temps indéfini et on pourra, de cette façon, déterminer la valeur pronostique de ces réactions.

	RÉACTION de Wassermann ordinaire (négative)	LUÉTINE- RÉACT.	RÉACTIVATION de la réaction de Wassermann
	mois		
1	16	—	—
2	21	—	—
3	24	—	—
4	24	—	—
5	24	—	—
6	15	+	+
7	17	+	+
8	18	+	+
9	19	+	+
10	?	+	+

GROUPE III.

Dans le groupe III, nous trouvons dix cas de syphilis dans lesquels cinq donnèrent une luétine-réaction positive et cinq un résultat négatif à la luétine-réaction et à la *réactivation* de la réaction de Wassermann. Nichols conclut que la luétine-réaction est aussi sensible, sinon plus, que la *réactivation* du Wassermann dans les cas de syphilis latente; et si, de plus, on compare la simplicité de la luétine-réaction avec la complexité relative de *réactivation* de la réaction de Wassermann, qui demande d'abord une injection de Salvarsan et ensuite l'exécution d'une réaction de fixation de complément, on voit que la luétine-réaction a une valeur pratique considérable.

Les premières observations faites aussi bien par Cohen, Orleman-Robinson, Stoll,¹ Gradwohl, Nobl et Fluss, Kämmerer, sur la spécificité de la luétine-réaction ont été confirmées par les recherches récentes de Rytina, Wolfsohn, Schmitter, Baermann et Heinemann, Foster, Geber, Bellantoni, Fagliuoli et Fisichella, Atkinson Wood,

Brown, Pusey, Fordyce, Desneux, Loewenstein, Nicholson, Simpson, et d'autres. Baermann et Heinemann ont trouvé qu'un extrait préparé au moyen du spirochète de la maladie connue sous le nom de Yaws ou Framboesia a des effets semblables à ceux de la luétine, et que les deux donnaient la même réaction sur la peau des syphilitiques ou des malades atteints de Framboesia. On peut penser qu'il existe une réaction de groupe pour les organismes de deux maladies qui sont si rapprochées l'une de l'autre.

Disons un mot de la susceptibilité particulière et exagérée de la peau de personnes atteintes de syphilis tertiaire. Neisser a déjà fait constater ce fait. D'après mon expérience, j'ai constaté cet état d'irritabilité spéciale sur environ 10 p. 100 des cas de syphilis tertiaire, mais cet état ne prête en aucune façon à confusion avec la luétine-réaction, car cette réaction n'est pas nécessairement associée à une susceptibilité spéciale. En réalité, la peau de la majorité des syphilitiques qui donnent une luétine-réaction typique ne réagit en aucune façon à des injections de contrôle dans lesquelles on n'a mis aucun extrait de tréponème.

En résumé, la luétine-réaction nous donne un moyen de déceler l'existence d'infections syphilitiques à un stade chronique ou latent dans lequel les symptômes cliniques ou la réaction de Wassermann sont temporairement absents. Une *luétine-réaction* négative dans les cas chroniques de syphilis où les symptômes sont graves et la réaction de Wassermann évidente, indique un pronostic défavorable; tel est par exemple souvent le cas chez les individus atteints de paralysie générale. La *luétine-réaction* ne doit pas être mise en parallèle avec la réaction de Wassermann.

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Figure 1. La cuti-réaction à la luétine, la forme papuleuse dans un cas secondaire traité.



Figure 2. La cuti-réaction à la luétine, la forme pustuleuse dans un cas tertiaire latent.

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RESISTANCE TO A TUMOR-PRODUCING AGENT AS DISTINCT FROM RESISTANCE TO THE IMPLANTED TUMOR CELLS.

OBSERVATIONS WITH A SARCOMA OF THE FOWL.*

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PLATE 32.

No one of the problems opened to experiment by the transplantation of animal tumors has received more attention than that of the nature of resistance to these growths. The exact manner of the resistance, or resistances,—for there may well be several kinds,—is still undetermined; nevertheless one important fact has emerged, namely, that the fate of implanted tumor, as indicating this resistance, is influenced for the most part by the same conditions that influence the fate of implanted normal tissue. The conditions at the site of the graft's implantation, the age of the host, its health, species, even variety, and its relationship by blood to the previous host,—these and other factors influence in like manner engrafted tissue of both sorts.¹ Furthermore, resistance to transplanted tumor can be induced by a preceding injection of living tissue,² and whether this tissue be normal or neoplastic makes little difference.³ By the same means too animals can be made resistant against implanted normal tissue which ordinarily would grow in them, for example embryonic tissue, so that it now fails to develop (Fichera, Rous). The numerous data thus far obtained go to show that the

* Received for publication, June 21, 1913.

¹ Loeb, L., *Proc. Am. Phil. Soc.*, 1908, xlvii, 3. Fichera, G., *Arch. ed atti d. Soc. ital. di Chir.*, 1909, xxii, 1. Rous, P., *Jour. Exper. Med.*, 1910, xii, 344.

² Ehrlich, P., *Arb. a. d. k. Inst. f. exper. Therap.*, 1906, No. 1, 77.

³ Bashford, E. F., Cramer, W., and Murray, J. A., *Brit. Med. Jour.*, 1906, i, 207. Schöne, G., *München. med. Wchnschr.*, 1906, liii, 2517.

resistance which in some individuals prevents the growth of implanted tumor is a resistance directed against the graft as strange tissue, and is unconnected with the neoplastic qualities which this tissue happens to possess.

These are the findings with transplanted neoplasms. Of the specific factors concerned in resistance to the growth of spontaneous tumors, as distinct from non-specific factors, such as infection, mechanical disturbance, etc., practically nothing is known. Manifestly these growths cannot be looked upon as consisting of a tissue strange to the body in the sense that transplanted tumor tissue is strange. The not infrequent occurrence of spontaneous tumors in animals highly resistant to implanted growths (Bashford) proves this.

The present paper is a report of an attempt to learn, through the study of an avian tumor, something of the nature of resistance to spontaneous growths. The tumor employed is a spindle-celled sarcoma of the chicken, which has already been reported upon several times from this laboratory.⁴ The growth, when transmitted by transplantation, behaves exactly like the transplantable tumors of mammals, being influenced by the factors which influence them in common with transplanted normal tissue. But from it there can be separated by drying, or filtration, or glycerinization, an agent, presumably living, which, under special conditions, will cause a sarcomatous change in the tissue of a previously normal fowl. The growth thus generated is derived from the host's own tissues and in this regard resembles the spontaneous tumors of mammals. A study of the forces influencing such a growth's development, and the circumstances which sometimes bring about its retrogression may well have a bearing on similar problems in mammalian oncology.

Natural retrogression of the spontaneous tumors of mammals is relatively rare; but retrogression can be induced, more or less successfully, by the Roentgen rays, radium, and ultraviolet light. The point of attack of these forms of radiant energy is still unknown. In the case of the chicken sarcoma when influenced by radiant

⁴ Rous, P., *Jour. Exper. Med.*, 1910, xii, 696; for references to other articles on the growth, see Rous, P., and Murphy, J. B., *Berl. klin. Wchnschr.*, 1913, 1, 637.

energy there arises at once the question whether the sarcomatous cells as such are primarily affected, or whether the agent responsible for the growth is injured, with a resultant lessened malignancy of the cells so that they can be destroyed by the body. My first experiments have been directed to this matter. Certain differences in the time of origin of growths caused by the dried tumor tissue, that is to say by an action of the tumor-producing agent, and of those resulting from the fresh tissue containing transplantable tumor cells have suggested the method of work.

METHOD.

With parallel safety-razor blades the fresh sarcomatous tissue is cut into a number of slices of equal thickness, ordinarily 0.08 to 0.1 cm. These are placed in Ringer's solution, carefully trimmed free of all except sound tumor, and are then separated into a number of batches. If the tissue is to be submitted to the Roentgen ray the slices of each batch, spread flat, are together sealed in a large hollow-ground slide under a large, thin cover-glass. If the ultraviolet ray is to be used the slices are repeatedly washed with Ringer's solution to rid them as far as possible of free albuminous matter, and, covered with a thin layer of the fluid, are exposed to the light in an open dish; or without the fluid they are sealed between two flat pieces of quartz glass previous to exposure. The control is treated in the same way except that it is not irradiated. Radium has not been employed thus far.

When the exposures have been completed the tissue is cut fine with sterile knives. In some of the experiments a little sterile infusorial earth was added to it. By means of trocars small portions (about 0.01 c.c. each) are now inoculated intradermally in the feather-free pectoral strip of a number of chickens. The remainder of the tissue is spread very thin in a dish, and placed *in vacuo* over sulphuric acid for twenty-four hours, which is sufficient time to render it completely dry. It is then restored to approximately its former bulk by the addition of an excess of Ringer's solution, or a very little distilled water, and inoculations are made with it as on the preceding day, using the same chickens and the feather-free strip of the other breast. Usually five batches of irradiated tissue and a control batch have been used, fresh and dry, making in all twelve inoculations to each fowl. The tissue bits are implanted several centimeters apart, in a line, and their order is varied from fowl to fowl. The tumors resulting are extremely discrete and are plainly visible at all stages of their development.

An Heraeus mercury-quartz lamp of 220 volts supplied the ultraviolet rays. The specimens were exposed at 25 cm. distance. The temperature of the preparations was at no time above 28° C.

For the Roentgen rays one or another of three soft tubes with a spark gap of 1.5 to 4.5 cm. has been employed. The specimens were placed within 5 to 10 cm. of the tube.

Five sets of observations with the ultraviolet rays and four with the Roentgen rays have been made by this method. For each experiment three to seven fowls were used, making in all forty-one inoculated, of which forty developed tumors. Retrogression soon occurred in some.

The growths from the inoculation of fresh material in intradermal sites arise in general almost immediately, resulting, as previous work has shown,⁵ from an extremely rapid proliferation of the implanted cells. At the end of a week the little tumors may be one centimeter in diameter. They appear as raised, translucent bosses or buttons over which the epidermis is tense and smooth (figures 1 and 2). At the end of two or three weeks they very commonly ulcerate, or coalesce, so that observations beyond this period are not of great value. The growths from the dried material are exactly similar but appear much later, not until at least seven days have elapsed, and often twice or thrice this time. That desiccation completely kills the tumor cells seems certain from the findings of previous workers with the normal and neoplastic cells of the higher animals. Moreover, experiments by Dr. Murphy show that the embryonic tissue of the fowl does not survive drying; and drying renders completely innocuous another transplantable chicken tumor (Chicken Tumor XVIII), propagated in this laboratory.

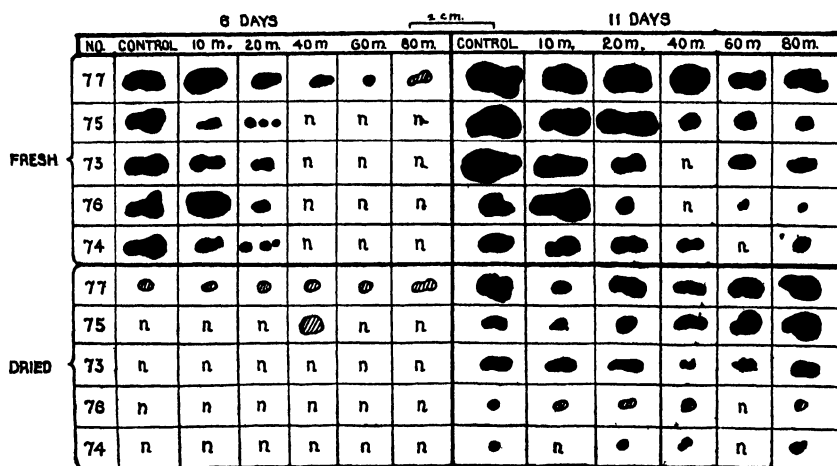
EFFECTS OF THE IRRADIATION.

The experiments have given almost diagrammatic results (text-figures 1 to 6). The Roentgen rays do not appreciably affect either the sarcoma cells or the growth's causative agent when the tissue has been exposed for eighty minutes, the longest time employed. This failure to be influenced is not surprising when it is considered how refractory many mammalian sarcomata are to the rays. The protocols of the individual experiments will not be given.

Ultraviolet light rapidly destroys the activity of the sarcoma cells and this without notably injuring the agent associated with them. In text-figure 1 its selective action is shown. At the end of six days good sized growths are found as the result of inoculation of the fresh control material, while the irradiated fresh tissue mani-

⁵ Rous, P., and Murphy, J. B., *Jour. Exper. Med.*, 1912, xv, 270.

fects less tumor-producing activity, until with sixty and eighty minutes' exposure this is completely suppressed. The dried material has as yet given rise to no growths. At the end of eleven days the effects of irradiation and of drying are less marked. In four of the five fowls the dried material has given rise to growths; and in the size of these growths there is no evidence of influence of the irradiation. Differences among the tumors from the fresh material still

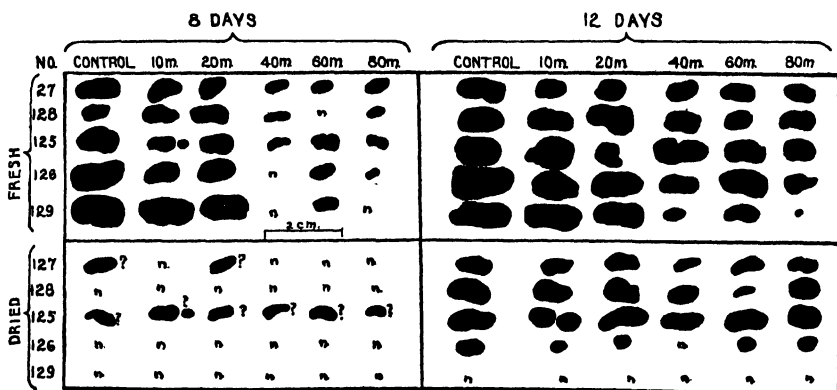


TEXT-FIG. 1. To show the effect of ultraviolet light on the activity of fresh and dried tumor tissue. In the first vertical column are the numbers designating the fowls. Then follow silhouettes to scale of the tumors. The measurements were taken six and eleven days after the inoculations. 10 m., 20 m., 40 m., etc. = 10, 20, and 40 minutes' exposure; n = no tumor. Cross-hatching = induration.

It will be seen that the irradiation has a marked injurious effect on the element in the fresh tissue (the living tumor cells) which gives rise to tumors immediately after implantation. The tumor-producing agent which resists drying is unaffected by the irradiation.

indicate this influence, but less strikingly than before, since now at length the material irradiated for sixty and eighty minutes has given rise to growths. These growths have arisen after about the same time and are of about the same size as those derived from the dried material. In text-figure 2 similar findings are presented but the differences are not so clear cut, since the longest period of irradiation has failed to rid the fresh tissue of the activity specifically associated with its fresh state.

The interpretation of these results is plain. The text-figures show, first, that in the sarcomatous tissue there are two elements capable of producing the growth, one of which will withstand drying while the other will not. The labile element, which we know to be the living and transplantable tumor cells, is so sensitive to the ultraviolet rays that sixty to eighty minutes' exposure will completely destroy its activity; whereas the stable element, the tumor-producing agent, is at most only slightly affected by this irradiation.



TEXT-FIG. 2. This chart is arranged in the same way and shows findings similar to those of text-figure 1.

TYPES OF RESISTANCE TO THE SARCOMA.

Taken from another point of view the irradiation experiments have demonstrated the presence in the fowl of two distinct resistances to the chicken sarcoma, directed, the one against the tumor cells, the other against the tumor-producing agent. These may exist separately or in one host.

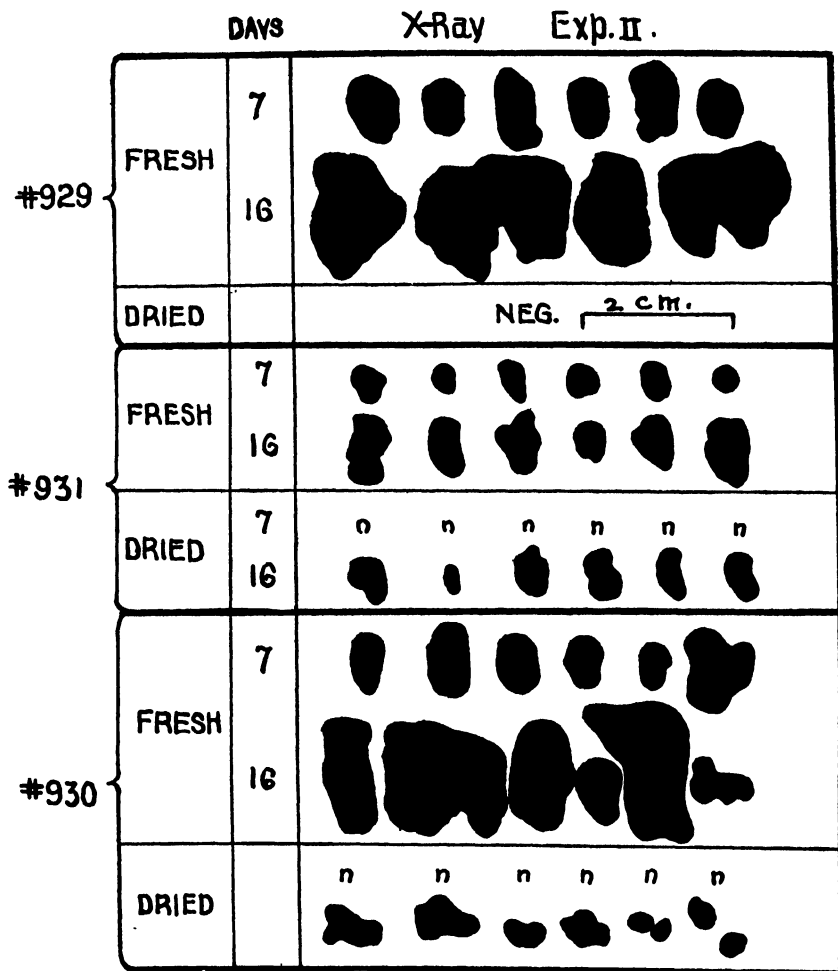
In text-figure 3 are given the findings in three of the fowls of text-figure 2 but now so arranged as to bring into contrast the fate of the fresh and dried material in the individual host. The fresh material produced tumors in all three fowls but with a very different degree of success. In one instance, No. 129, in which it gave rise to large tumors the dried material failed to engender growths, and in another, No. 126, this latter acted only slowly. In the remaining

instance growths developed rapidly from the dried material and the fresh material proved only slightly more active, the results with it being poor as compared with those in the first two hosts. In text-figure 4 precisely the same facts are shown of some fowls of X-ray experiment II, and here the complicating effect of a variation in tumor size due to injury from the irradiation is not

ULTRAVIOLET		Exp. VI	CONTROL	10 m.	20m	40m.	60m	80m.
#129	FRESH	8 DAYS				n		n
		12 DAYS						
	DRIED	8 DAYS	NEG.					
		12 DAYS						
#126	FRESH	8 DAYS						
		12 DAYS						
	DRIED	8 DAYS	n	n	n	n	n	n
		12 DAYS						
#127	FRESH	8 DAYS						
		12 DAYS						
	DRIED	8 DAYS		?		?		
		12 DAYS						

TEXT-FIG. 3. Some of the results shown in text-figure 2, but now so arranged as to demonstrate individual differences in the resistance of the fowls and the presence of two sorts of resistance. In No. 129 the fresh tissue rapidly gave rise to tumors, whereas the same material, when dried, failed to engender them. That this is not due to lack of activity on the part of the dried stuff is shown by the findings in the other two fowls. In fowl 126 the fresh tissue rapidly engendered tumors and the dried material in due time caused them. In No. 127 the dried material was active in the production of growths, whereas the fresh material was much less successful than in the fowls previously mentioned. It is evident that some hosts have a relative resistance against a tumor-producing element in the fresh tissue, while in some a resistance is directed against the tumor-producing element which survives drying. ? = possibly a tumor, possibly only induration.

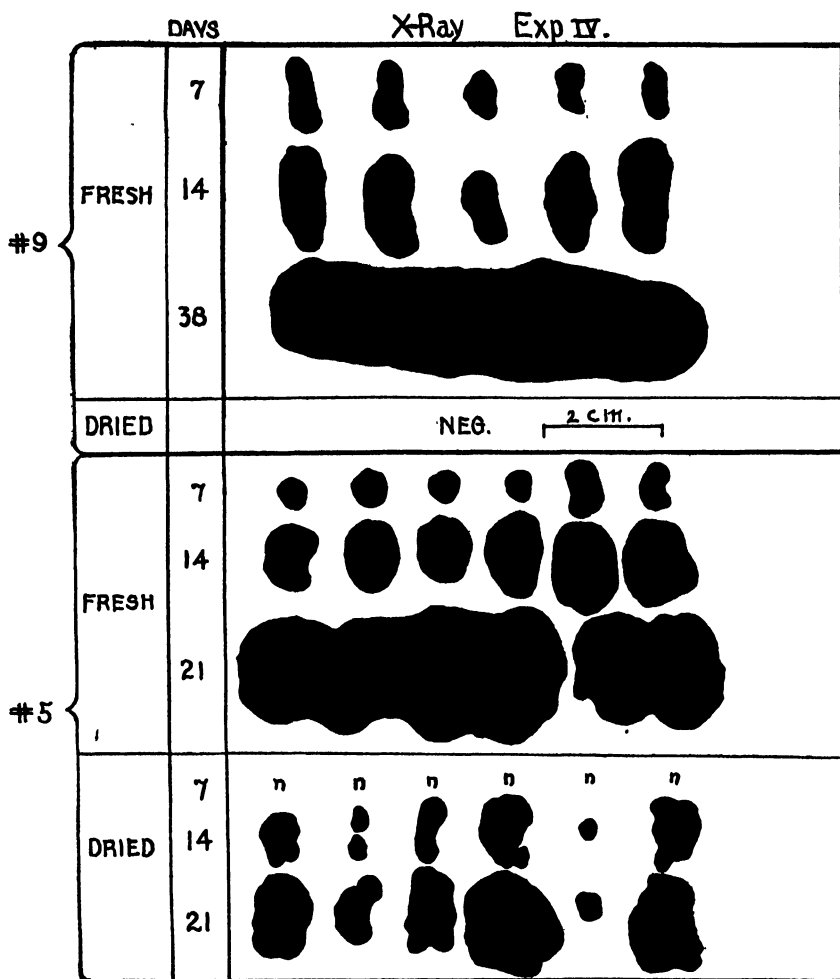
present. In text-figures 5 and 6 some phases of the matter are again illustrated. The fowls in which the dried material is very active



TEXT-FIG. 4. This illustrates the same facts as text-figure 3. The periods of exposure to the X-rays are not given since the material was practically unaffected by them.

are not necessarily those in which the fresh material does badly, but often ones in which it does very well. Other charts showing the same facts might be given. The chickens of all the experiments

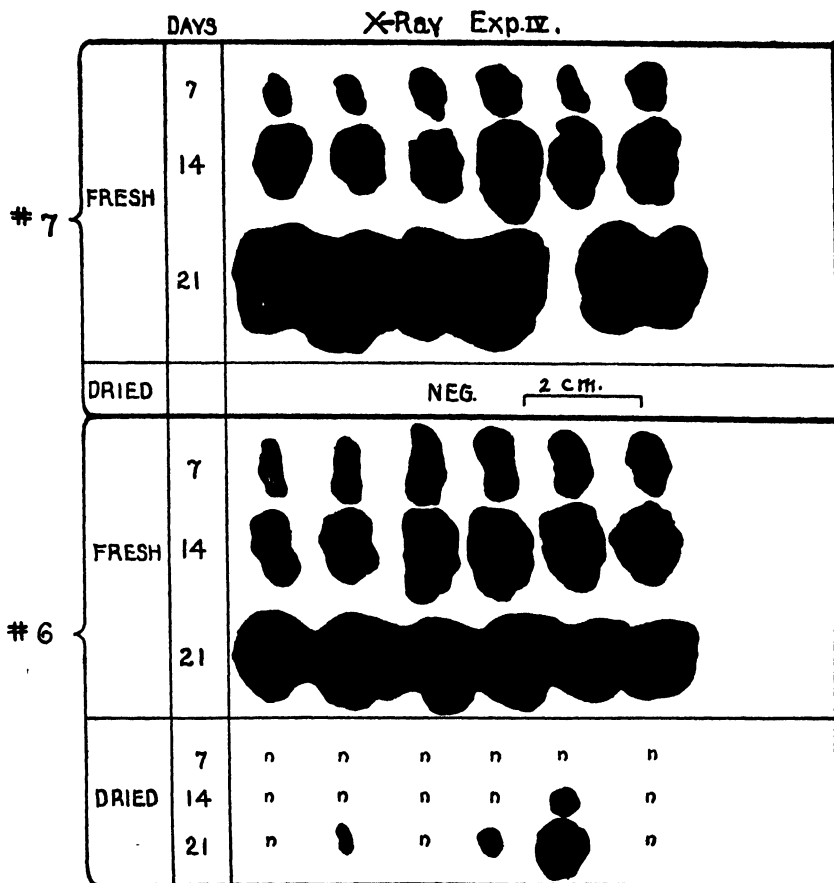
can be separated into four classes: those in which the fresh material gave rise to tumors and the dried gave none; those in which both



TEXT-FIG. 5. This illustrates further some of the facts of text-figures 3 and 4 and shows that good growth from dried material is not necessarily associated with poor results from the fresh tissue.

gave rise to tumors with nearly equal facility; others again in which neither gave rise to growths; and finally a majority in which both

inoculations resulted in growths, those from the fresh material appearing earlier and maintaining a marked general advantage of size. In other words the chickens showed themselves resistant or susceptible in widely various degrees to one, or another, or both the



TEXT-FIG. 6. This chart shows again the differences in individual fowls.

labile and stable elements going to produce the tumor. And we know that these labile and stable elements are, respectively, the tumor cell and the tumor-causing agent.

A number of questions at once present themselves in relation to this conclusion. Resistance to the transplanted tumor cells is never

so clear cut as that to the agent. It is at best only relative. If fowls are really resistant to the implanted cells how does it happen that even in hosts most resistant to them growths from the fresh material appear somewhat earlier and at first are larger than those from the dried material? One reason is because in resistant fowls a profuse, local, round-celled reaction occurs about the fresh tissue implanted in intradermal sites, forming transiently a considerable nodule.⁶ Perhaps also the tumor-producing agent is somewhat attenuated by drying. Theoretically the findings, in fact all of the differences between fresh and dried material, might be explained on the assumption that the chicken sarcoma is never really transplanted but comes only from infection by means of an agent largely impaired by drying. This is contrary to the facts and entails awkward secondary assumptions, for example, that fowls react differently to the stable and labile portions of the agent, some being susceptible only to the one, some to the other. But we know that the differences between fresh and dried material are primarily those of the tumor cells involved. Furthermore, investigations have shown that some fowls are naturally resistant to the action of the tumor-producing agent⁷ and some to growth of the implanted tumor cells.⁸ The present work goes only a step further in demonstrating that the resistances thus manifested are independent of one another.

It is interesting to consider in the light of these results the rôle of the causative agent in the growth of the sarcoma in the individual fowl. Histologically there is no suggestion that the agent takes part in this process. And yet in one class of susceptible fowls, as the present results show, it doubtless aids in the growth's extension. In another class the tumor's development following the implantation of tumor cells is probably from the first solely the result of the proliferation of these cells.

In the present state of our knowledge it is impossible to say whether findings with chicken tumors have a direct application to

⁶ Rous, P., and Murphy, J. B., *Jour. Exper. Med.*, 1912, xv, 270.

⁷ Rous, P., Murphy, J. B., and Tytler, W. H., *Jour. Am. Med. Assn.*, 1912, lviii, 1751.

⁸ Rous, P., *Jour. Exper. Med.*, 1910, xii, 696; Rous, P., and Murphy, J. B., *Berl. klin. Wchnschr.*, *loc. cit.*



FIG. 1.



FIG. 2.

(Rous' Resistance to Tumor-Producing Agent.)

the problems of mammalian tumors. Were this so then the present observations would go far to explain how it happens that "spontaneous" growths can arise, as they sometimes do, in rats or mice highly resistant to transplanted neoplasms. For in these individuals resistance to implanted cells need by no means carry with it resistance to a tumor-producing agent.

SUMMARY.

Ultraviolet light rapidly kills the cells of a transplantable sarcoma of the fowl without notably injuring the etiological agent associated therewith. The Roentgen ray has little effect on either cells or agent.

Fowls manifest two sorts of resistance to the avian tumor, one directed against the implanted tumor cells as such, the other against the action of the etiological agent to cause a neoplastic change. In the individual fowl the two resistances appear to be independent of one another, though they may exist together or may both be absent. A recognition of them will perhaps explain some features in the biology of other tumors.

The work has been done with the assistance of Dr. Linda Lange.

EXPLANATION OF PLATE 32.

FIG. 1. A photograph taken eight days after the implantation of material exposed to the Roentgen rays, showing the discrete character of the intradermal tumors. Tumors have arisen, thus far, only from the fresh material. The dried tissue was put in the left pectoral strip. The large mass to the left of the upper end of the sternum is the crop.

FIG. 2. Intradermal tumors in the pectoral strips. The preparation has been turned so that the sternal keel, if present, would lie horizontally along its middle.

The upper line of growths (right pectoral strip) has resulted from the inoculation of fresh tumor tissue exposed in a glass tube to the ultraviolet light for various periods. In the lower line are growths from some of the same material exposed in the same way but in a quartz tube. At the extreme left of each line (left of the photograph) is a tumor from the control material. The periods of irradiation become longer as one proceeds to the right. The photograph was taken on the seventeenth day after the inoculations.

It will be seen that the ultraviolet rays have failed to act through glass, but through quartz their action has been such that at the end of seventeen days tumors are just beginning to appear from the material irradiated longest (fifteen and thirty minutes).

EXPERIMENTS ON THE CULTIVATION OF THE MICROÖRGANISM CAUSING EPIDEMIC POLIOMYELITIS.*

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PLATES 38 TO 41.

Several attempts have been made to demonstrate under the microscope and to develop in artificial cultures the microörganism causing epidemic poliomyelitis, but hitherto unsuccessfully. Giersvold¹ cultivated certain micrococci from the cerebrospinal fluid, and Fox² a bacillus from the circulating blood of poliomyelitic patients, but both have now been discredited as causes of the disease. The discovery of the filterable nature of the causative agent independently by Flexner and Lewis³ and Landsteiner and Levaditi⁴ not only disposed finally of the claims made for the above mentioned bacteria, but also discouraged renewed efforts at cultivation. Flexner and Lewis⁵ noted and Levaditi⁶ confirmed a clouding of serum bouillon by an aqueous Berkefeld filtrate of the central nervous tissues of poliomyelitic monkeys, but the phenomenon proved to be due to protein precipitation and not to multiplication of a living parasite. Proescher⁷ has recently stained certain coccus-like bodies in films prepared from the central nervous organs of monkeys infected experimentally with the virus of poliomyelitis. The precise nature of these bodies has still to be worked out; ap-

* Received for publication, June 20, 1913.

¹ Giersvold, M., *Norsk Mag. f. Lægevidensk.*, 1905, iii, 1280.

² Dixon, S. G., Fox, H., and Rucker, J. B., *Pennsylvania Department of Health, Laboratory Report*, March 2, 1911.

³ Flexner, S., and Lewis, P. A., *Jour. Am. Med. Assn.*, 1909, liii, 2095.

⁴ Landsteiner, K., and Levaditi, C., *Compt. rend. Soc. de biol.*, 1909, lxvii, 592.

⁵ Flexner, S., and Lewis, P. A., *Jour. Am. Med. Assn.*, 1910, liv, 45.

⁶ Levaditi, C., *Presse méd.*, 1910, xviii, 44.

⁷ Proescher, F., *New York Med. Jour.*, 1913, xcvi, 741.

parently they have not yet been demonstrated in human nervous tissues, and similar bodies have been found in the nervous organs of animals experimentally infected with rabic materials. The comparatively large size of many of these coccoid bodies leaves it doubtful whether they can bear relation to filterable viruses.

The successful cultivation of spirochætæ, both parasitic and saprophytic, by Noguchi⁸ provided the impulse for a renewed attempt at the cultivation of the microörganismal cause of poliomyelitis. The application by Noguchi of his method to the central nervous tissues derived both from human and from experimental (monkey) examples of poliomyelitis has yielded results of an entirely different order from those previously obtained. The results have already been described briefly in our Fifteenth Note⁹ and they will be described more fully in this place.

NATURE AND SOURCES OF MATERIALS.

During the several years that epidemic poliomyelitis has prevailed in the eastern United States we have collected many specimens from affected human beings and monkeys. Portions of nervous and other organs were regularly set aside in 50 per cent. sterile glycerin in the refrigerator. Hence there were available for the cultivation tests both fresh specimens yielded by recent autopsies upon human beings and monkeys and material preserved for periods ranging from a few days to many months. The new experiments upon cultivation were begun during the summer of 1912, at which time many acute cases of poliomyelitis were being cared for in the Hospital of the Rockefeller Institute, where a number of autopsies came to be performed upon fatal cases. Thus the cultivation was carried out simultaneously upon fresh, sterile, or practically sterile (for ordinary bacteria) specimens derived from human beings and from monkeys experimentally inoculated, and upon glycerinated specimens from both sources.

The material subjected to cultivation should be taken as soon after the death of the person or animal as possible, and should be obtained when possible in an aseptic condition. While any part

⁸ Noguchi, H., *Jour. Exper. Med.*, 1911, xiv, 99; 1912, xv, 90; xvi, 199, 211.

⁹ Flexner, S., and Noguchi, H., *Jour. Am. Med. Assn.*, 1913, lx, 362.

of the central nervous system is suitable, the brain is to be preferred for the reason that it can be obtained more readily than the spinal cord or the intervertebral ganglia in an aseptic state. The dura covering one of the hemispheres having been seared with a hot instrument is incised with sterile instruments and the underlying cortex scrupulously exposed. From this a piece of about two cubic centimeters extending into the white matter is excised and placed at once into a sterile dish. If desired, portions of the cerebellum may be obtained in a similar manner, and by removing the cerebral hemispheres and then lifting up the cerebellum the medulla may be exposed in an aseptic manner, so that portions may also be taken.

It is advisable to make the initial inoculation with fragments as well as with emulsions or with filtrates of the nervous tissues. The fragments are to be preferred since they require almost no manipulation. When, however, the original material is suspected or known to be contaminated, it is advisable to employ filtrates at the same time. The filtrates are prepared by grinding the nervous tissue in strength of about 5 per cent. with sand in distilled water or physiological salt solution. The resulting emulsion is shaken in the machine for about thirty minutes, after which the suspended matter is removed by centrifugalization and the supernatant fluid is pressed through a Berkefeld filter, N. or V.

METHOD OF CULTIVATION.

The culture medium consists essentially of human ascitic fluid, to which has been added a fragment of sterile fresh tissue. In contradistinction to the spirochætæ, for which sheep serum water supplies a suitable medium, the microörganism isolated from the nervous organs of cases of poliomyelitis demands ascitic fluid. In obtaining the initial culture, the exclusion of oxygen is necessary, but it is not, as in the case of *Treponema pallidum*, essential that the inoculated tubes should be placed in an anaerobic jar; it suffices to cover the liquid with a deep layer of sterile paraffin oil. In some instances, however, the initial growth can be obtained more certainly when the anaerobic jar is employed.

Test-tubes measuring 1.5 by 20 centimeters are chosen. Into

each of a series a fragment of the usual size of sterile fresh kidney of the normal rabbit is placed. Next is added a fragment of corresponding size of the cerebrum or other part of the brain or spinal cord. Upon these are poured about fifteen cubic centimeters of sterile ascitic fluid and finally about four cubic centimeters of the sterile paraffin oil. The ascitic fluid must have been originally sterile, since sterilization by fractional heating or filtration renders it unsuitable. The control tubes consist of the ascitic fluid and kidney fragment without brain tissue and ascitic fluid plus brain tissue without the kidney fragment. It is best to prepare two sets of the cultures, one to be placed in the anaerobic jar and the other kept outside. The incubation is conducted at the temperature of the thermostat, namely, at 37° C.

The culture tubes in the anaerobic jars should not be disturbed for from seven to twelve days, while those not within the jar may be inspected daily. Any tube showing marked turbidity, coagulation, or gas production within one or two days may be set aside as grossly contaminated. The tubes which remain clear or show only slight turbidity may none the less be contaminated. To exclude contamination small quantities of the medium are removed with pipettes and stained for bacteria in the ordinary way and cultivated upon the usual solid or fluid media. Obvious bacteria in the films as well as any growth taking place in the ordinary media in the presence of air may be put down as contaminations and the tubes containing them discarded.

Gross contaminations having been excluded, the peculiar micro-organism obtained from the poliomyelitic nervous tissues exhibits the following growth characters. At the expiration of about five days' incubation a faint opalescence appears about the fragments of tissue at the bottom of the tube. This opalescence can be gradually diffused through the tube by gentle shaking (agitation), in the course of which it is observed that the turbidity about the tissues was really greater than was at first apparent (figure 1, fluid culture). The control tubes when not grossly contaminated either remain perfectly clear or a slight granular precipitate of washed out particles of tissue forms about them. The two appearances are quite distinct and become more pronounced after another period of

three to five days, during which time the opalescence first described extends into the upper portion of the medium, while in the controls the precipitate gathers more and more at the lower end of the tubes. At the expiration of ten or twelve days the diffuse opalescence of the medium in the tubes in which growth has been observed to take place begins to diminish as sedimentation sets in, during which minute, irregular particles form, heavy enough to fall slowly to the bottom of the tube.

The corresponding tubes contained in the anaerobic jar when examined on the seventh day show a similar opalescent growth but slighter than that just described. When replaced in the jar and returned to the thermostat the opalescence increases so that at the expiration of another period of five or six days it approximates that present at the end of one week in the tubes kept outside the jar.

Cultivation may also be performed in a solid medium, consisting of ascitic fluid and sterile rabbit tissue, to which a suitable quantity of 2 per cent. nutrient agar has been added in order to produce a solid mixture. The melted agar, cooled to a temperature of 40° C., is mixed with the ascitic fluid in the tube containing the fragment of tissue, also previously heated to 40° C. After cooling and solidification the medium should be sufficiently firm to maintain its stability. In this medium an initial growth, aside from contaminations, from poliomyelitic material has never been obtained, but once growth has been secured in the fluid medium it is possible, but not in all instances, to transmit it to the solid medium. Often successful transfer from the fluid to the solid medium is not accomplished until about the third generation of the growth has been obtained in the fluid medium. The changes indicative of growth in the solid medium begin about the fragment of tissue and appear first as a diffuse opalescence which gradually aggregates into visible grains of minute size that are recognized as colonies. Several days are usually required before this initial change in the medium becomes apparent. The rapidity with which it does become apparent is affected by the generation of the culture and hence by its adaptation to the medium. Gradually the opalescence rises in the medium within the tube, until it reaches within about three centimeters of the surface of the medium, at which a sharp line of demarcation

appears. When the number of colonies developing is large the individuals are so minute as to give a diffuse grayish appearance to the medium, which requires a lens to bring out the separate colonies. When the number of colonies developing is smaller they may reach a diameter of about one third of a millimeter and hence become visible as such to the naked eye, to which they appear round in shape and grayish white in color. Under favorable conditions the opalescence indicating growth about the tissue fragment becomes apparent at the end of forty-eight hours and distinct after seventy-two to ninety-six hours, although several days may be required before the ascent of the growth to the line of demarcation, at which the presence of oxygen brings about a cessation of development (figure 2, solid culture).

The description just given relates to cultivation with fragments of fresh central nervous organs in the ascitic fluid and in the presence of sterile rabbit tissue. It is, however, possible to secure cultivation even in the absence of the rabbit tissue. For this purpose a somewhat larger fragment of the poliomyelitic brain is placed in the tubes containing the ascitic fluid, which is then covered with the layer of paraffin oil. After incubation at 37° C. the initial growth takes place, but with less constancy than when the rabbit tissue is also present. When it occurs the growth is usually so slight that the opalescence remains absent or nearly so, and it becomes necessary to transplant into a medium containing fresh rabbit tissue to establish its existence.

Just as it is possible to eliminate the fresh rabbit tissue so is it possible to dispense with the ascitic fluid, which may be substituted by an extract prepared from the brain tissue or even sheep serum water. The extract is prepared by grinding in a porcelain mill and then afterwards shaking for half an hour in a machine twenty grams of fresh normal brain of a *Macacus rhesus* monkey in 500 cubic centimeters of distilled water or Ringer solution. The emulsion is centrifugalized and the supernatant fluid passed through a Berkefeld filter, V. The clear sterile filtrate is used to replace the ascitic fluid, but in order to obtain growth in this fluid or in serum water it is necessary to employ a fragment of rabbit tissue. A successful result has never been obtained with either, in the absence of the

rabbit tissue, from which it follows that they are inferior to the ascitic fluid as a medium of cultivation.

In several parallel experiments an emulsion of the brain tissue was compared with the brain fragment as the source of the culture and it was found that growth was obtained with greater difficulty and somewhat less frequency from the emulsion than from the whole tissue fragment. While growth was detected during the first week in the corresponding tubes of fragment and emulsion, a week later the growth may have disappeared from the emulsion tubes, from which it was concluded that certain constituents of the nervous tissue, liberated by the emulsion, exerted an injurious effect upon the developing microörganism. In support of this supposition it was found that when an emulsion or extract of the brain was added to an active culture prepared in the usual way the micro-organisms previously readily demonstrable in stained preparations became indistinguishable. That they do not entirely disappear under these circumstances is shown by the fact that when transplanted into the ascitic fluid tissue medium a growth reappears.

The Berkefeld filtrates of emulsified tissue also give rise to cultures, but less constantly than the original nervous material, from which the filtrates were prepared. In cultivating with the filtered extract, the ascitic fluid rabbit tissue medium should always be employed, and two sets of tubes prepared, one to be placed in the anaerobic jar and the other to be kept outside. The initial appearance of growth is somewhat delayed, so that an average of ten days of incubation may be necessary before the characteristic alteration of the medium becomes apparent. In no instance was success attained with the filtrate in the absence of the rabbit tissue.

Glycerinated human and monkey poliomyelitic tissues were employed as fragments, and, in the case of monkeys, as filtrates for cultivation. The specimens had been kept in 50 per cent. glycerin at a temperature of 2 to 4° C. for periods varying from twenty-five days to one year. Portions of the brain or spinal cord so preserved were cut off with sterile scissors, washed thoroughly in sterile distilled water, and placed in the ascitic fluid tissue medium. The resulting growths, when they occurred, produced the characteristic appearances already noted. On the whole, the glycer-

inated tissues gave somewhat more inconstant results than the fresh material.

Once the initial growth from any of the sources mentioned has been secured it is, as a rule, possible to obtain subcultures by transplanting about 0.2 of a cubic centimeter of the original culture per tube into the next series. Since the initial growth begins about the tissue at the bottom of the tube it is advisable to take the fluid for transplantation from this location. Subcultures are obtained more constantly in the fluid than in the solid medium. Hence growth will sometimes appear in the former and not in the latter. Once the microorganism has been adapted to the conditions of artificial cultivation, transplantation to the solid medium may be effected. The adaptation is indicated by a more rapid and abundant growth in the fluid medium, and its occurrence is promoted by the presence of fresh sterile tissue and is prejudiced by its absence. Among the monkey tissues was one specimen which had been glycerinated for nine months, and from which a growth was obtained with a Berkefeld filtrate.

In the course of the growth the sterile kidney turns first pink and then gray in color, but no obvious solution or hemolysis of the red corpuscles contained in the tissue occurs.

The addition of various carbohydrates and alcohols, such as glucose, saccharose, lactose, maltose, gelactose, levulose, mannite, glycogen, dextrin, inulin, dulcite, and arabinose, to the ascitic fluid or sheep serum water in proportion of 0.5 per cent. did not affect the cultivation, and the growths when pure had no apparent effect upon them, since neither acid nor gas are produced.

Litmus milk, to which fragments of the sterile fresh tissue have been added, affords a medium in which growth will take place without producing any alteration in the appearance of the medium.

NUMERICAL RESULTS OF CULTIVATION.

In a series of thirty-three experiments with the nervous tissue of monkeys, comprising thirteen specimens of brain tissue, two of brain emulsion, and eighteen filtrates of brain and spinal cord from twenty-four animals infected from two different strains of human poliomyelitic virus, an initial growth was obtained in nineteen in-

stances, of which sixteen proved to be pure and three mixed cultures. Of these, pure subcultures were obtained thirteen times, namely, eight times from brain tissues, four times from filtrates, and once only from emulsion. In a second series in which human nervous tissues were directly employed, eight successful cultivations were secured. One was from fresh tissue, three were from recently glycerinated tissues, and four from tissues which had been glycerinated for many months. In two specimens of the last four, initial cultures but no subcultures were obtained. In all the others subcultures resulted and were maintained alive for an indefinite period.

MORPHOLOGICAL CHARACTERS.

Uncontaminated cultures of the microörganism from the various human and monkey sources already mentioned possess common morphological and staining characters. Fluid cultures viewed under the dark-field microscope exhibit among the innumerable dancing protein and other granules present minute bodies, globular in form, hanging together in short chains, pairs, and small masses, devoid of independent motility and distinguishable with difficulty as a special class among the indefinite granules present. Stained preparations, on the other hand, bring out unmistakable micro-organisms grouped in the three ways stated and of very minute size. Staining may be accomplished with more or less difficulty in several ways, but the two methods which have thus far yielded the most satisfactory results are those of Giemsa and Gram.

The Giemsa stain may be applied in two ways according as a rapid or a somewhat slower but better result is desired. In the first, the film, either upon a slide or a cover-glass, is allowed to become air-dry, when it is fixed over the flame, and several drops of Giemsa solution are dropped upon it and diluted with about twenty drops of distilled water. After gentle mixing of the dye and water the preparation is held over the flame until a trace of vapor rises. Care is exercised to avoid boiling. The preparation is then washed in a stream of distilled water, pressed between blotters, and dried. This method does not yield uniform staining or constancy of shade, but is useful for rapid examinations. The second method is the one usually employed. The film having been air-dried is

fixed in methyl alcohol for about an hour, washed in distilled water, and is then immersed in the solution containing one drop of the stain per cubic centimeter of distilled water, in which the film remains for from two hours to twelve to fourteen hours (over night). Gram's stain is applied in the usual way, using gentian violet as the dye and safranin as the counterstain. The microorganism behaves variably towards the Gram stain. When the medium in which it is grown contains peptone, it retains the stain more assiduously than when it is devoid of that substance. Then the intensity with which the Gram stain is retained varies with the age of the culture. The older the culture the more strongly the gentian violet is held. While the grouping is of course identical whichever stain is employed, the apparent size of the microorganism is affected by the dye. When the gentian violet is retained the individuals appear larger than they do with either the safranin or the Giemsa stain. Loeffler's alkaline methylene-blue, carbol-fuchsin, and carbol-gentian violet all stain the microorganism slightly or without affording sharp differentiation. The microscopical appearances of the microorganism vary somewhat according to whether the growth is in a fluid or solid medium and peptone is present or absent. Peptone and possibly other constituents of the bouillon render the microorganisms somewhat larger as well as enable them to retain more firmly the Gram stain.

Films prepared from the lower layer of the ascitic fluid culture about the sterile rabbit tissue and stained by Giemsa solution reveal the presence of a variable number of minute globoid bodies, arranged in pairs or short chains, or in small aggregated masses. In the fluid culture the pairs and chains predominate (figures 3, 4, and 5). According as the staining operation was carried out by the rapid or slower method, the globoid bodies assume a bluish or a violet color; rarely they present a red tint. Since the most uniform results are obtained by the slower method of staining, it may be considered that the violet coloration is the normal. The individual microorganisms average about 0.2 of a micron in diameter, the limits of visible bodies being 0.15 to 0.3 of a micron. Possibly still smaller individuals exist that exceed the limit of visibility. For purposes of comparison a group of chain forms has been compared by photography with *Streptococcus pyogenes* (figure 6).

Considerable difficulties may be encountered in demonstrating the microörganism in initial cultures not older than four or five days. Either nothing whatever that can be recognized as microörganisms may be detected, or merely roundish bodies the nature of which it may be difficult to decide. When the same tubes are examined on the sixth or seventh day the unmistakable minute globoid bodies are usually detected. Although the original growth tends to appear slowly, the final multiplication takes place rapidly, and is completed from about the eighth to the twelfth day, after which the cultures remain stationary for a variable period. Kept in the thermostat the microörganisms may remain largely unchanged for many days, and in the refrigerator for many weeks.

Within the solid cultures the microörganism develops not as chains but as pairs and aggregated groups. The arrangement is very readily ascertained by compressing a fragment of the agar between two slides, which are afterwards separated. The resulting films having been stained in the usual manner by Giemsa or Gram solution, the minute particles of agar adhering to the glass will frequently exhibit colonies, of which the members are sufficiently separated to expose the grouping (figure 7). In the Gram-stained films the bodies are somewhat coarser than in the Giemsa-stained ones. In young agar cultures the Gram-stained bodies are partly purple and partly pink, while in older cultures almost all appear purple.

After a somewhat variable period, and generally from ten days to three or four weeks after the maximum growth has been obtained, certain metamorphoses or degenerations set in. Probably the enlarged and irregular stained bodies are to be viewed as degenerations, while the minute fragments just at the limit of visibility may possibly represent a metamorphosis into a minuter variety with which submicroscopic forms may be associated. When the conditions of culture are unfavorable, as, for example, when the brain extract or other medium without sterile tissue is employed, this supposed metamorphosis appears to arise earlier, and indeed within the first few days of growth. Subcultivation from one of the altered cultures or from their Berkefeld filtrates yields frequently but not uniformly new developments of the visible microörganisms.

The important question, as to whether the cultures just mentioned contain actual ultramicroscopic forms of the microörganism, or whether the failure to detect the visible forms is due chiefly to dilution, must remain for the present undecided. What is certain, however, is that the globoid organisms are capable of passing through Berkefeld filters which exclude the usual test bacteria (*e. g.*, *Micrococcus prodigiosus*). Cultures from the Berkefeld filtrates should always be made in the ascitic fluid tissue medium, as in the case of the initial growth.

The film preparations stained by the Giemsa stain and especially by the rapid method sometimes show the globoid organisms surrounded by a zone either faintly tinted or colorless, that may equal in width the diameter of the individual microörganisms. Single bodies, aggregated groups, or chains may be thus enveloped. The nature of the material composing the zone is unknown, as is also whether it represents an independent part of the organism (capsule?) or is derived from the culture medium.

INFECTION OF MONKEYS.

The microörganism described is present so constantly in the central nervous tissues of affected human beings and monkeys that a strong presumption exists to the effect that it bears some relationship to epidemic poliomyelitis. It is sufficiently obvious that whatever they may ultimately prove to be, as far as we now know the globoid bodies are not common pathogenic or even saprophytic microörganisms. Whether the bodies are limited to the central nervous organs or occur more widely distributed in the body of infected human beings and monkeys is still to be determined. The question which presses at the moment is whether the cultures are capable of setting up in monkeys clinical and pathological states corresponding with those characteristic of experimental poliomyelitis. Before proceeding to describe the inoculation experiments, it may be well to point out that apparently the globoid bodies are highly parasitic microörganisms. In view of this circumstance it may readily happen that they may quickly lose pathogenic properties in artificial cultures. There are of course several instances known among the bacteria and spirochætæ of which this circum-

stance is true. We were therefore prepared to encounter difficulties in the way of infecting monkeys with cultures. It is necessary to recall the difficulties surrounding the original implantation of highly infected human poliomyelitic material upon monkeys in order to have presented emphatically the probable obstacles that stand in the way of the successful accomplishment of the infection of monkeys with the cultures.

Two series of inoculations were made. In one the cultures derived from the nervous tissues of monkeys and in the other from human beings directly were separately inoculated into *Macacus rhesus* monkeys. The inoculations were made either into the brain or into the sciatic nerve and peritoneal cavity simultaneously.

Before describing the several instances of successful inoculation, certain important points should be presented. It is known that the virus in an emulsion or filtrate prepared from the nervous tissues of infected monkeys survives in the thermostat and remains infectious for at least thirty days, and, also, that the filtrate soon becomes inactive after being transferred to a new fluid medium consisting of serum bouillon. Possibly the inactivity arises chiefly from the great dilution which the virus has undergone. Whence it follows that dilutions of the virus beyond the point at which infection through intracerebral inoculation of several cubic centimeters of suspension can be produced are easily secured. We have frequently had occasion to confirm this general fact, since second transfers into the fluid medium from a supposed culture derived from monkeys previously inoculated with the highly active M A virus have in many instances failed to cause infection even when injected intracerebrally. Dilution beyond the point of infectivity of the virus, even in the culture media, thus is readily accomplished.

Bearing these facts in mind it follows that successful inoculation with culture generations still more remote than the second dilution of the original virus is to be interpreted as representing, probably, an implantation of the artificially cultivated microorganism upon the susceptible monkeys. Moreover, from the monkeys in which experimental poliomyelitis has been thus produced the microorganism introduced can be recovered in culture and even implanted successfully again.

The implantation upon monkeys of the virus contained in the original human nervous tissues is admittedly difficult. When filtrates are inoculated failure usually follows. When emulsions are employed, infection occurs in from about 50 to 100 per cent. of the instances, probably depending upon variation in the degree of infectivity of the original virus. The human strains of the virus are less infectious for monkeys than the adapted or so called monkey strains, a fact that is the more significant as respects the successful inoculation of cultures derived directly from human tissues.

Cultivated Human Strains.—Fluid and solid cultures in the third generation from four human sources, namely, D., Kr., Ko., and F., were mixed. Each culture had been obtained from recently glycerinated specimens of the central nervous system.

Experiment A.—Macacus rhesus. December 27, 1912. 0.5 c.c. of the mixture was injected into the left sciatic nerve and 0.8 c.c. into the left cerebral hemisphere.

December 30, 1912. Animal less active than normally; legs and arms weak.

December 31, 1912. Excitable; uses legs awkwardly. Etherized.

Autopsy.—At the site of intracerebral inoculation punctiform hemorrhages; the left lateral ventricle contains a small, dark clot; the cervical, dorsal, and lumbar spinal cord appear congested; the left sciatic nerve is slightly swollen at site of injection. Film preparations from the two inoculation sites show no ordinary bacteria. The microscopical examination of the medulla, cervical, dorsal, and lumbar levels of the spinal cord, and intervertebral ganglia from the cervical and lumbar regions shows in all typical and marked characteristic lesions of experimental poliomyelitis. The lesions consist of degeneration of the nerve cells, associated with neurophagocytosis, infiltration with mononuclear cells of the ground substance, and perivascular and meningeal infiltration (figures 8, 9, and 10).

Experiment B.—Macacus rhesus. December 31, 1912. A suspension of the medulla and spinal cord of monkey A was injected into left sciatic nerve and left cerebral hemisphere.

January 1 to January 4, 1913. Increased excitability.

January 6, 1913. Excitable; vision defective; ataxia.

January 7, 1913. Arms weak, right more than left.

January 8, 1913. 9.30 A.M., prostrate; respiratory paralysis. Death at 10 A.M.

Autopsy.—A small cyst with clear contents at the site of injection. Sciatic wound healed. Congestion of gray substance of spinal cord.

The microscopical examination exhibits marked lesions of experimental poliomyelitis in the spinal cord, medulla, and intervertebral ganglia. Portions of the nervous system were placed in 50 per cent. sterile glycerin.

Experiment C.—Macacus rhesus. May 14, 1913. Inoculated intracerebrally with emulsion of glycerinated spinal cord and medulla of monkey B.

May 18, 1913. Excitable.

May 19, 1913. Arms weak.

May 20, 1913. Arms and legs paralyzed.

May 21, 1913. Etherized.

Autopsy.—A small cyst at the site of inoculation. At various levels of the spinal cord are visible lesions.

Microscopical examination shows marked and characteristic lesions of experimental poliomyelitis in the medulla, spinal cord, and intervertebral ganglia.

Cultures in the ascitic fluid tissue medium were made at autopsy from monkeys A, B, and C. From monkeys A and B the original microörganism injected was recovered in pure culture. The culture from monkey C was unsuccessful. Hence another portion of the original glycerinated material was inoculated as follows:

Experiment D.—*Macacus rhesus*. June 7, 1913. Intracerebral injection of emulsion of glycerinated brain and cord from monkey B.

June 12, 1913. Excitable; tremor; prostrate.

June 13, 1913. Etherized.

Autopsy.—A small cyst at the site of inoculation; visible lesions in the spinal cord.

Microscopical examination shows typical lesions of experimental poliomyelitis in the medulla, spinal cord, and intervertebral ganglia.

Cultures in the ascitic fluid tissue medium from this animal gave a pure growth of the microörganism originally injected into monkey A, the beginning of this series, more than six months previously. The successful culture in this instance indicates that the failure to recover the microörganism from monkey C was due to a technical fault.

Cultivated Monkey Strains.—These strains consisted of cultivations from the highly adapted M A virus which had been long propagated in monkeys from emulsions or filtrates of the nervous organs. Hence the cultures, while representing the same original virus, varied in precise generation according to the period at which they were isolated. All were far removed from the original human strain.

Experiment E.—*Macacus rhesus*. December 3, 1912. Two cultivated strains, one in the third and the other in the fifth generation, were combined, and of the mixture 1 c.c. was injected into each sciatic nerve, and 10 c.c. were injected into the peritoneal cavity.

December 6, 1912. Excitable; coat roughened.

December 9, 1912. Somewhat less excitable.

December 23, 1912. Again excitable. The monkey remained excitable continuously, and on March 19, 1913, was found in convulsions, which recurred at frequent intervals, until on March 25 death occurred.

The microscopical examination reveals focal lesions in the medulla, consisting of minute hemorrhages, small aggregations of mononuclear cells, partly in the ground substance and partly about the small vessels, and a few small vascular thrombi. Small hemorrhages also occur in the crura cerebri, and in the cervical region of the spinal cord, in which slight perivascular infiltration with mononuclear cells is also present.

Cultures yielded the original microörganism.

The interpretation of experiment E is not perfectly clear. The long intervening period between the inoculation and the development of convulsions and occurrence of death is not strictly paralleled by the effects of direct inoculation of either emulsion or filtrate of the nervous tissues. Moreover, thrombi do not occur in uncomplicated instances of experimental poliomyelitis. On the other hand the infiltrative lesions are similar to those present in experimental poliomyelitis.

Experiment F.—Macacus rhesus. December 27, 1912. Injected into left cerebral hemisphere 0.8 c.c. of cultivated virus, fifth generation.

January 1, 1913. Excitable; arms weak.

January 2, 1913. Excitability continues; arms and back partly paralyzed; tremor of head.

January 3, 1913. Legs weak.

January 4, 1913. Animal prostrate. Etherized.

Autopsy.—A small cyst at the site of injection. Spinal cord edematous. No visceral lesions.

Microscopical examination shows marked characteristic degenerative and infiltrative lesions of experimental poliomyelitis in the medulla, spinal cord, and intervertebral ganglia.

Cultures yielded the original microörganism.

Experiment G.—Macacus rhesus. January 27, 1913. Injected into left cerebral hemisphere 2.5 c.c. of culture in fifth generation from monkey E.

February 1, 1913. Excitable; movements uncertain; vision appears to be defective.

February 2, 1913. Neck and back paralyzed; unable to sit up; convulsions in course of which death occurs.

Autopsy.—February 3, 1913. The general viscera are apparently normal. A small cyst the size of an almond is present at the point of inoculation in left hemisphere. Gray matter of medulla and spinal cord is congested.

The microscopical examination of the medulla, spinal cord, and intervertebral ganglia shows typical lesions of experimental poliomyelitis.

Cultures yielded the original microörganism.

Experiment H.—Macacus cynomolgus. February 5, 1913. Injected into the left cerebral hemisphere 2.5 c.c. of a Berkefeld filtrate prepared from the site of inoculation of monkey G.

February 6, 1913. Shows tendency to incline toward right side; does not use the right arm, which is spastic, the result probably of injury from the injection.

February 7, 1913. Death at 10 A.M.

Autopsy.—At the site of inoculation there is a cyst containing blood stained fluid communicating with the lateral ventricle.

Microscopical examination shows marked lesions in the intervertebral ganglia and lesions of the blood vessels adjacent to the site of inoculation resembling those characteristic of experimental poliomyelitis. The spinal cord also reveals fine lesions of the same nature. Hence it appears that within the short period of forty-eight hours the specific lesions developed throughout a wide area of the nervous system of this animal.

Experiment I.—*Macacus rhesus*. February 8, 1913. Repetition of experiment H. Injected into the left cerebral hemisphere 1 c.c. of filtrate from site of inoculation of monkey F.

February 14, 1913. Prostrate; legs, back, and arms paralyzed. Etherized.

Autopsy.—A small, blood-containing cyst at the site of inoculation. Spinal cord throughout shows gross lesions of the gray matter.

The microscopical examination confirms the character of these lesions. Spinal cord, intervertebral ganglia, and medulla all show marked degenerative and infiltrative lesions of poliomyelitis, and identical lesions occur in the crura cerebri and in the cerebral hemispheres.

Experiment J.—*Macacus rhesus*. February 13, 1913. Suspension of glycerinated brain and spinal cord of monkey F injected intracerebrally.

February 18, 1913. Death.

Autopsy.—A cyst the size of an almond containing clotted blood occurs at the site of inoculation. Throughout the spinal cord the gray matter is congested.

Microscopical examination shows marked characteristic lesions of experimental poliomyelitis.

Experiment K.—*Macacus rhesus*. May 14, 1913. Injected intracerebrally with an emulsion of glycerinated spinal cord and medulla obtained from monkey F.

May 18, 1913. Distinctly weak.

May 19, 1913. Ataxic.

May 20, 1913. Paralysis of arms, legs, and back.

May 21, 1913. Etherized.

Autopsy.—At the site of inoculation there is a small cyst containing blood. Visible lesions in the spinal cord.

Microscopic examination reveals marked characteristic poliomyelitic lesions of the medulla, spinal cord, and intervertebral ganglia.

Culture yielded microorganism identical with that inoculated in monkey F almost six months previously. This culture in the fourth generation was inoculated intracerebrally on June 11, into a *Macacus rhesus*. On June 16 the monkey was weak, slow, and ataxic; it was etherized on July 2. The microscope reveals typical lesions of the brain and spinal ganglia, or, in other words, evidences of the encephalitic type of poliomyelitis.

Experiment L.—*Macacus rhesus*. January 13, 1913. Injected into left cerebral hemisphere 1 c.c. of cultivated virus, sixth generation.

January 16, 1913. Movements irregular; tends to move in circles.

January 17, 1913. Apparently normal.

February 6, 1913. Ataxic; excitable, arms and legs weak.

February 10, 1913. Weakness of arms and legs increased. Etherized.

Autopsy.—No lesion at the site of inoculation, and no gross changes in the spinal cord.

Microscopical examination shows fine infiltrative and degenerative lesions of the medulla, spinal cord, brain, and intervertebral ganglia.

Culture yielded microorganisms identical with that inoculated.

Experiment M.—Macacus rhesus. February 11, 1913. Injected emulsion of the spinal cord and brain of monkey K into the sciatic nerve and peritoneal cavity.

February 14, 1913. Excitable; convulsions.

February 15, 1913. Legs weak.

March 1, 1913. Excitability continues; movements slow and restricted.

March 5, 1913. Condition unchanged. Etherized.

Microscopical examination shows typical poliomyelitic lesions of the intervertebral ganglia in the lumbar and cervical regions, and similar but inconspicuous lesions of the spinal cord.

Experiment N.—Macacus rhesus. February 27, 1913. Inoculated into left hemisphere with 2 c.c. of monkey culture from two sources in the eighteenth and twentieth generation respectively.

March 1, 1913. Inclines slightly towards the right side.

March 2, 1913. Death.

Autopsy.—At the site of inoculation is a cyst the size of an almond containing clear fluid and communicating with the lateral ventricle; gray matter of the spinal cord is congested.

Microscopical examination of the cyst shows the presence of a Gram-positive coccus (mixed infection).

Experiment O.—Macacus cynomolgus. March 4, 1913. 2 c.c. of Berkefeld filtrate from the site of inoculation of monkey N are injected intracerebrally, and 100 c.c. intraperitoneally.

March 17, 1913. Left arm weak; legs somewhat spastic.

March 18, 1913. All extremities weak and spastic.

March 19, 1913. Head retracted; convulsions; unable to rise. Etherized.

Autopsy.—No lesion at the site of inoculation. A *placque jaune* 1.5 by 2 cm. occupies the surface of the cortex on the left side, posterior to the motor area. General viscera normal.

Microscopical examination reveals characteristic focal lesions of experimental poliomyelitis in the intervertebral ganglia, and vascular and interstitial lesions of the spinal cord, crura cerebri, and cerebral hemispheres.

Because of contamination with micrococci at the inoculation site in monkey N, a filtrate prepared from the local site was injected into monkey O, resulting in combined flaccid and spastic paralysis. However, the sections of the nervous tissues of monkey O by showing lesions of experimental poliomyelitis indicate the existence of that disease.

The preceding experiments show unmistakably that the inoculation of the cultures is followed by the appearance of the clinical symptoms and pathological effects characteristic of experimental poliomyelitis in the monkey. The two methods of inoculation now chiefly practised with the usual suspensions or filtrates of nervous tissues were employed successfully with the cultures. The essential results are identical whether the cultures are inoculated into the brain or simultaneously into sciatic nerve and peritoneal cavity. The inoculation of the cultures produces in monkeys no direct effect. Immediately after injection the animals appear normal and they remain apparently in this condition for an incubation period which may be as brief as forty-eight hours, but commonly endures for one to several weeks, at the expiration of which the usual symptoms of excitement, tremor, weak and then paralyzed muscles succeed one another. Death may arise from prostration, convulsions, or paralysis of the respiration, although in most of the instances related it was artificially hastened by etherization.

The central nervous tissues of the monkeys may be employed for recultivation of the microörganism inoculated, or for transmitting the experimental affection to still other and probably an indefinite series of monkeys. This transmission can be accomplished either with emulsions or Berkefeld filtrates of the fresh or glycerinated organs. Since the experimental disease is accurately reproduced in this manner, it follows that not only the cultures but the nervous tissues infected with them possess the distinctive disease-producing properties of the virus of poliomyelitis as commonly understood.

The local effects of the cultures vary with the inoculation site and the dosage. Unless secondary infection arises from the outside, the intrasciatic and intraperitoneal inoculations cause no considerable reaction, while the intracerebral injection leads to the formation of a small cyst, in which the introduced microörganism may or may not be readily discoverable in film preparations. If relatively few of the microörganisms are inoculated, they tend to disappear from view; while if many are introduced, they are readily detected, either lying free or enclosed in emigrated polynuclear or mononuclear cells (figure 11). When pyogenic micrococci enter from the skin, the local lesion tends to be larger, and to contain

many pus cells, and the two microörganisms may appear side by side, both without and within cells. When an excess of the mixed fluid and solid cultures is injected into the brain, the local effect is also more severe and many leucocytes may be called out by the positively chemotactic elements contained within the medium. The action of contaminated cultures is less readily interpreted than are the effects of pure cultures; but any doubt may be removed by preparing a Berkefeld filtrate from the local lesion and portions of the medulla, spinal cord, and intervertebral ganglia, which is to be employed for inoculation.

The disappearance of the microörganism from the inconspicuous local lesions probably results from a general diffusion throughout the nervous organs and restricted multiplication at the site of injection. The microörganism can indeed be recovered in cultures from any part of the central nervous system, and is best recovered from the intact cerebral hemisphere, where it usually exists in a pure state and is readily accessible under aseptic conditions.

RELATION OF THE MICROÖRGANISM TO NERVOUS TISSUES.

The microörganism the cultures of which have been described can be detected in film preparations and in sections prepared from the central nervous organs of human beings who have succumbed to poliomyelitis and of monkeys subjected to inoculation with the ordinary virus, or with cultures. A special technique which has been devised by Noguchi is required for the demonstration.

Staining Method for Film Impressions.—1. Cover-glasses of suitable size and thinness (No. 1 or 0) receive a succession of impressions from the selected piece of nervous tissue.

2. After becoming air-dry the cover-glass is placed, film side down, in a mixture which is freshly prepared at each operation, consisting of one part of Grüber's Giemsa solution, and two parts of Merck's methyl alcohol reagent, where it remains for two minutes. In order to prevent the film surface from contact with the bottom of the staining dish, it is well to support one end upon a drawn out glass capillary tube. The staining vessel may conveniently be a small Petri dish and cover.

3. Twenty parts of a 1 to 10,000 potassium hydrate solution are

poured into the dish at the expiration of the two minutes, and the whole is thoroughly mixed by gentle agitation. At the expiration of one hour the cover-glass is removed.

4. After brief washing in distilled water, the sections are rapidly passed in succession through acetone, acetone seventy parts plus xylol thirty parts. This operation requires several seconds and careful observation.

5. The cover-glass is now washed in distilled water for two minutes, dried in the air, and mounted in cedar oil.

Staining Method for Sections.—The nervous tissues having been hardened in Zenker's solution or sublimate alcohol are embedded in paraffin and the sections are mounted upon slides in small series.

1. The sections are treated with Lugol's solution and afterwards with a 0.5 per cent. solution of sodium hyposulphite, after which they are thoroughly washed in distilled water.

2. The sections are passed successively through 95 per cent. alcohol, absolute alcohol, chloroform, acetone, benzol, ether, absolute alcohol, 95 per cent. alcohol, and finally distilled water, in each of which reagents they remain about one hour.

3. The staining solution consists of three cubic centimeters of concentrated Giemsa solution to forty-five cubic centimeters of distilled water, in which the sections remain for twenty-four hours.

4. After brief washing in distilled water, the sections are rapidly passed in succession through acetone, acetone seventy parts plus xylol thirty parts, acetone fifty parts plus xylol fifty parts, acetone thirty parts plus xylol seventy parts, and finally pure xylol.

5. The cleared sections are mounted in cedar oil.

With these methods the minute microörganism has been demonstrated in the films in eighteen out of nineteen specimens of glycerinated human nervous tissues. It was detected regularly lying between cells and in some instances within the cytoplasm of cells, some of which were nerve cells (figures 12 and 13). The microörganism appears invariably in pairs or small aggregated groups and never in chains. The demonstration of the microörganism in sections is far more difficult, but it has been accomplished in a few instances in the interstices of the tissues and in the protoplasm of cells.

The detection of the microörganism in film preparations and in sections has been accomplished also with nervous tissues derived from monkeys inoculated at one time with the ordinary virus, at another with cultures. In this case also it is more readily brought out in film preparations than in sections. The grouping is identical with that present in the specimens of human origin.

DISCUSSION.

From the facts presented it follows that by employing a specially devised method there has been cultivated from the central nervous tissues of human beings and monkeys the subjects of epidemic poliomyelitis a peculiar minute organism that has been caused to reproduce the symptoms and lesions of experimental poliomyelitis. The microörganism consists of globoid bodies measuring from 0.15 to 0.3 of a micron in diameter, and arranged in pairs, chains, and masses, according to the conditions of growth and multiplication. The chain formation takes place in a fluid medium, the other groupings in both solid and fluid media. Within the tissues of infected human beings and animals the chains do not appear.

No statement is ventured at present as to the place among living things to which the bodies belong. It is obvious that the cultural conditions are those that apply more particularly to the bacteria.

On the other hand, the microörganism is associated with the production of an acute disease in which suppuration does not form a prominent part. No special attention at the present time has been given to the solution of the question as to whether the micro-organism actually belongs to the bacteria or to the protozoa. In the manner of evolution of the symptoms, and in the appearance of the lesions, the experimental disease caused by the inoculation of the cultures resembles that produced by the virus of poliomyelitis as ordinarily employed. The central nervous organs of monkeys infected with the cultures bear preservation and glycerinization as do the infected human tissues, or the monkey tissues infected directly from human tissues. Cultures to which glycerin is directly added survive in the refrigerator at least eight days.

The microörganism passes through Berkefeld filters and the

filtrates yield upon recultivation the particular microörganism contained within the filtered culture. Moreover, Berkefeld filtrates prepared from the nervous tissues of infected human beings and monkeys yield also in culture the identical microörganism.

By employing a suitable staining method the microörganism has been detected in film preparations and sections prepared from human nervous tissues, and from the corresponding tissues of monkeys inoculated with the usual virus or with cultures or filtrates prepared from monkeys previously injected with cultures. From all the infected materials mentioned, irrespective of the manner of their origin, the microörganism has been recovered in cultures. As would be expected it is more uniformly recoverable from the original nervous tissues than from filtrates, and doubtless for the reason that in the former it exists in greater abundance.

To obtain the initial culture is difficult, and this irrespective of whether the tissues submitted to cultivation have come immediately from man or from monkeys previously inoculated with the ordinary virus or even with the cultures. Once the microörganism adapts itself to a parasitic state it is developed with greater difficulty under saprophytic conditions. Whenever the nervous tissues have been shown to be infectious, the microörganism has been recoverable, notwithstanding long preservation and glycerination. In other words, infectivity of the nervous organs and the presence of the microörganism exist together. It has indeed happened that a specimen of infected nervous tissue has at the first attempt not yielded the initial growth, although it has yielded it upon the second attempt. Persistence will usually lead to a successful cultivation, provided no technical fault is committed. An important factor in the technique of cultivation is the sample of ascitic fluid. Not all samples are suitable, and a preliminary test is necessary, using for the purpose a growing culture, in selecting samples for culture purposes. Once a suitable ascitic fluid is obtained it should be carefully husbanded in the refrigerator. Even with this precaution failure may still occur. In such an instance repetition, using the same materials but in two series, one of which is prepared for enclosure in the anaerobic jar, while the other is allowed to remain outside, may yield the desired result; or the

result may come on a second trial that appears to be an exact repetition of the first.

Only the exceptional cultures possess the degree of pathogenicity sufficient to cause specific infection, and the production of experimental poliomyelitis. A pathogenic strain may be effective at different and even remote generations, and a non-pathogenic strain may lack pathogenicity even in the second generation. This important fact indicates strongly that the pathogenic effect is not due to mere mechanical carrying over into the cultures of an invisible parasite or virus with which the cultivated microörganism is accidentally associated. If such accidental association were the cause of the experimental disease produced by the cultures in monkeys, it would display itself preferably in the first generations and without reference to the strain of the visible microörganism. On the other hand in this fluctuation of pathogenicity the cultures imitate the action of the virus as contained in human materials, namely, nervous tissue, secretions from the nasopharynx, and intestinal washings, in which the virus, either known or believed to be present, may yet fail to be demonstrated by reason of the want of infectious power for monkeys or for the particular monkey inoculated in a given instance. Moreover, it is a common experience in bacteriology to find even among the ordinary bacteria lack or rapid loss of virulence among saprophytic cultures, while virulence is not only retained, but may be increased in rapid passages from animal to animal.

In view of these considerations it would appear that an etiological relationship has been shown to exist between the cultivated micro-organism and epidemic poliomyelitis as it occurs in human beings or in monkeys. There remains merely a single other possibility, already indicated, namely, that two factors are present in the cultures, the one an invisible because ultramicroscopic organism, the other the globoid bodies described. On this basis it would have to be supposed that the former but hypothetical factor is the essential agent of infection. As against this supposition it may be urged that an instance of symbiosis of this nature is not known to animal pathology. Regarding the cultivated minute but visible microörganism itself, it may be held on the basis of the data presented that it fulfills the conditions hitherto demanded for the

establishment of causal relation between an extraneous parasite and a specific disease. The microörganism exists in the infectious and diseased organs; it is not, as far as is known, a common saprophyte, or associated with any other pathological condition; it is capable of reproducing, on inoculation, the experimental disease in monkeys, from which animals it can be recovered in pure culture. And besides these classical requirements, the microörganism withstands preservation and glycerination as does the ordinary virus of poliomyelitis within the nervous organs. Finally, the anaerobic nature of the microörganism interposes no obstacle to its acceptance as the causative agent, since the living tissues are devoid of free oxygen and the virus of poliomyelitis has not yet been detected in the circulating blood or cerebrospinal fluid of human beings, in which the oxygen is less firmly bound; nor need it, even should the microörganism be found sometimes to survive in these fluids.

EXPLANATION OF PLATES.

PLATE 38.

FIG. 1. Fluid culture showing opalescence. Natural size.

FIG. 2. Solid culture showing colonies and line of demarcation. Natural size.

PLATE 39.

FIG. 3. Separate globoid bodies. $\times 1,000$.

FIG. 4. Aggregated masses of globoid bodies. $\times 1,000$.

FIG. 5. Chains and pairs of globoid bodies. $\times 1,000$.

FIG. 6. Chains of globoid bodies compared with *Streptococcus pyogenes*. $\times 1,000$.

FIG. 7. Agar fragment showing pairs of globoid bodies compared with *Streptococcus pyogenes*. $\times 1,000$.

PLATE 40.

FIG. 8. Spinal cord showing degeneration of nerve cells and invasion of leucocytes (neurophagocytosis). $\times 144$.

FIG. 9. Perivascular and interstitial mononuclear cell infiltration of medulla. $\times 98$.

PLATE 41.

FIG. 10. Interstitial infiltration and degeneration and phagocytosis of nerve cells in intervertebral ganglion. $\times 160$.

FIG. 11. Globoid bodies in site of inoculation taken up by leucocytes. $\times 1,000$.

FIG. 12. Film preparation from central nervous tissue of monkey inoculated with human virus (not culture) showing pairs of globoid bodies. $\times 1,000$.

FIG. 13. Section prepared from spinal cord of monkey inoculated with M A virus (not culture) showing intracellular pairs of globoid bodies. $\times 1,000$.

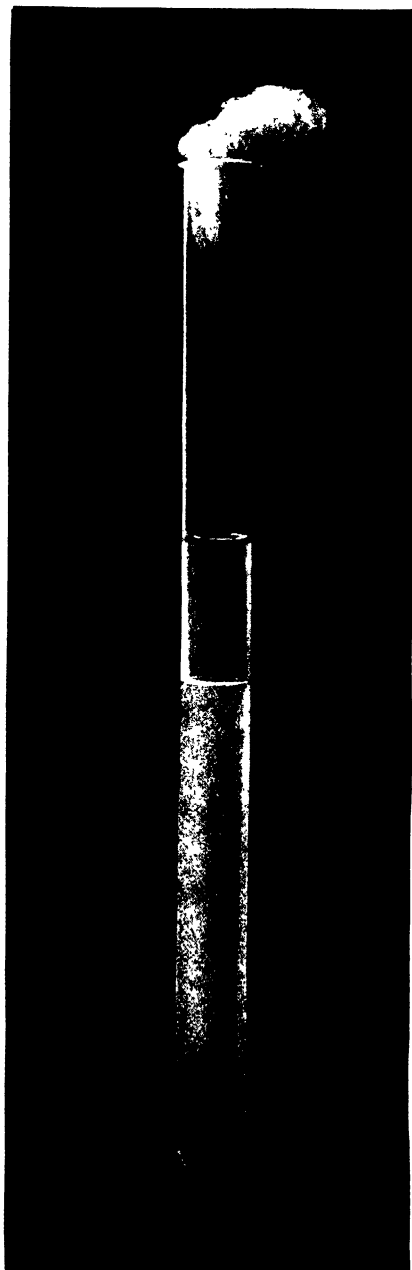
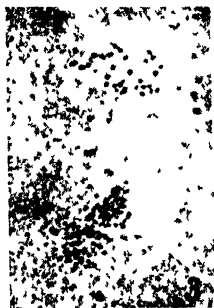


FIG. 1.



FIG. 2.

(Flexner and Noguchi: Microörganism Causing Epidemic Poliomyelitis.)



4



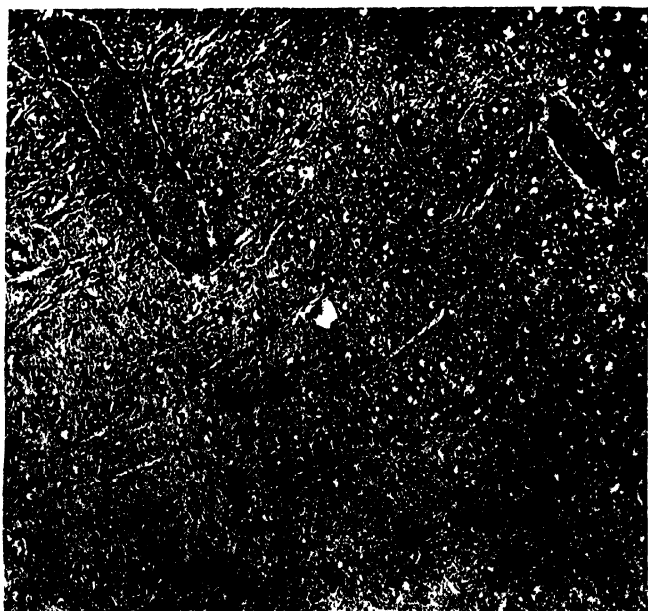
5



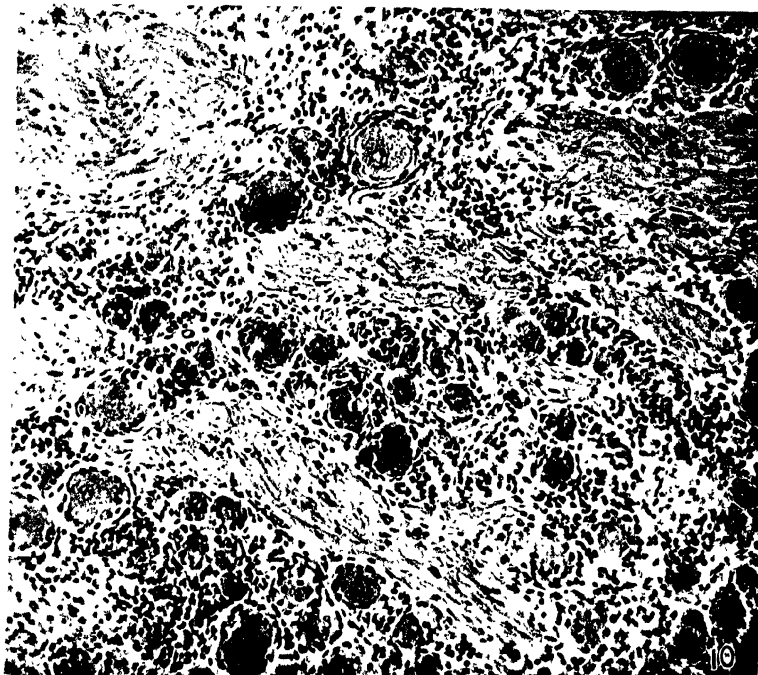
6



(Flexner and Noguchi: Microorganism Causing Epidemic Poliomyelitis.)



(Flexner and Noguchi: Microorganism Causing Epidemic Poliomyelitis.)



(Flexner and Noguchi: Microorganism Causing Epidemic Poliomyelitis.)

ÉTAT ACTUEL DE LA CULTURE DU TRÉPO- NÈME PÂLE.

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Depuis que Schereschewsky a annoncé en 1909 qu'il avait réussi à cultiver, en partant des tissus syphilitiques humains, certains spirochètes à l'état impur qu'il croyait être des spirochètes pâles, il a paru de différentes sources un grand nombre d'articles sur le même sujet. Jusqu'à ce jour, l'on compte au moins dix investigateurs qui prétendent avoir obtenu des cultures pures de spirochètes pâles: Mühlens, W. H. Hoffmann, Noguchi, Sowade, Arnheim, Tomaszewski, Shmamine, Nakano, Baeslack, et tout récemment encore Schereschewsky, et six autres qui n'ont obtenu que des produits impurs: Bruckner et Galasesco, Boas et Proca, Danila et Stroe.

Les milieux de culture employés par les différents investigateurs peuvent se diviser en trois groupes principaux, à savoir: *a*) du sérum de cheval, ou d'autres animaux, solidifié (Schereschewsky, Mühlens, W. H. Hoffmann, Tomaszewski, Sowade, Nakano, Shmamine, Bruckner et Galasesco, Boas); *b*) eau de sérum avec du tissu animal frais (Noguchi); et *c*) agar d'ascite, avec du tissu animal frais (Noguchi). Parmi ceux qui ont employé le milieu de Schereschewsky, Shmamine l'a modifié en y ajoutant une petite quantité de sodium nucléinique, Nakano de l'agar-peptone, et Proca et ses collaborateurs de l'acide pyrogallique. Baeslack s'est servi avec succès du milieu recommandé par moi.

La provenance des matériaux a varié. C'est ainsi que Schereschewsky, Sowade, Tomaszewski, Arnheim, Bruckner et Galasesco ont employé exclusivement ou principalement des tissus syphilitiques humains, tandis que Mühlens, Hoffmann, Shmamine, Nakano, Baeslack et moi-même, avons expérimenté avec des tissus syphilitiques tirés de l'homme aussi bien que du lapin. Toutefois il est

important de noter que Mühlens et d'autres ont eu un succès en se servant de lapins, tandis que j'ai pu obtenir six séries pures de dix sources différentes.

Les cultures ont été pratiquées par tous, à l'exception de Baeslack et de moi, sans précaution anaérobique stricte. Il est certain que les spirochètes cultivés par les premiers investigateurs peuvent se multiplier et se conserver pendant de nombreuses générations dans un sérum de cheval à moitié coagulé. Les spirochètes que j'ai isolés diffèrent des leurs en ce qu'ils ne peuvent exister sans l'élément de tissus frais et sans l'exclusion complète d'oxygène.

Ma méthode à l'eau de sérum et tissu est faite exclusivement pour les cultures obtenues en partant des lésions testiculaires de lapin, tandis que le milieu d'agar ascitique et de tissu ne convient qu'aux produits impurs tirés de matériaux syphilitiques humains; j'ai constaté que l'espèce de provenance humaine ne peut être cultivée dans le premier de ces milieux et que celle provenant du lapin ne se développe pas pendant la première génération dans un tissu avec agar ascitique, ni dans tout autre milieu connu (y compris le milieu de Schereschewsky avec ou sans addition de tissu stérile frais).

Ce fait a été confirmé par Tomaszewski.

En ce qui concerne la morphologie des spirochètes cultivés, différents investigateurs admettent que leurs cultures renfermaient à la fois les formes fines et les formes plus grossières, surtout lorsqu'il s'agissait de tréponèmes de provenance humaine. Schereschewsky, aussi bien que Mühlens, Shmamine et d'autres l'ont remarqué; Mühlens croyait que les deux formes appartenaient à des variétés différentes, tandis que Schereschewsky et Shmamine les tenaient pour une seule et même espèce se développant dans des conditions différentes. Personnellement, je partage l'opinion de Mühlens.

La première production réussie de lésions syphilitiques chez des animaux au moyen de cultures pures a été rapportée par moi en 1911; en me servant de deux séries obtenues du syphilome du lapin dans les testicules, j'ai provoqué des lésions testiculaires typiques chez plusieurs lapins. Par la suite, Hoffmann (1911), Sowade, Shmamine (1912), Nakano (1912) et Baeslack (1913) ont obtenu les mêmes résultats, tandis que Bruckner et Galasenco ont rapporté en 1910 un succès chez un lapin au moyen de leur matériel impur

(3^e génération). Tomaszewski (1912), ainsi que Sowade (1911), ont également réussi à infecter des lapins au moyen de cultures impures de provenance humaine. De plus, en 1912, j'ai provoqué des lésions typiques de la peau sur les sourcils de plusieurs singes (macaques et cercopithèques) au moyen de cultures pures provenant de tissus syphilitiques humains. La virulence de cet organisme disparut environ quatre mois après culture, tout en conservant ses autres propriétés biologiques intactes même au bout de presque trois ans.

Il existe des différences fondamentales en ce qui concerne les propriétés biologiques de l'organisme cultivé par Mühlens et Hoffmann d'une part, et moi-même de l'autre. Celui des premiers exhale une odeur de putréfaction et se multiplie, non seulement dans du sérum de cheval presque jusqu'à la surface, mais aussi dans l'agar pur et le bouillon pendant quelques générations en présence d'oxygène, tandis que le *Treponema* de mes cultures ne se développe que dans des conditions rigoureusement anaérobiques en présence de tissu stérile frais et ne produit jamais d'odeur désagréable. Après avoir été longtemps cultivés artificiellement, les spirochètes de mes cultures ne se développent pas dans le sérum de cheval de Schereschewsky, dans l'agar pur ou le bouillon. J'ai indiqué ces différences dans ma première publication. Plus tard, j'ai démontré que certains spirochètes de la bouche, *Treponema microdentium*, *Treponema mucosum*, présentaient en culture pure des caractères morphologiques qui ne peuvent être distingués de ceux des spirochètes pâles, mais qu'ils exhalaient une odeur de putréfaction caractéristique accompagnée d'altération du milieu de culture qui n'est pas sans quelque ressemblance avec celle décrite pour l'organisme de Mühlens et Hoffmann. Tomaszewski, Arnheim, Shmamine, Bae-slack et Nakano n'ont remarqué aucune odeur dans leurs cultures de spirochètes pâles. La culture de Mühlens, dénuée de pathogénicité dès le début, et celle d'Hoffmann, diffèrent totalement de mes cultures et de celles des investigateurs ultérieurs. Du moins, il n'est pas invraisemblable, comme l'a indiqué E. Hoffmann, que le fait que W.-H. Hoffmann a réussi à provoquer l'orchite syphilitique chez un lapin, peut résulter de ce fait qu'il s'est servi d'une culture impure de spirochètes pâles renfermant un spirochète étranger à odeur, ou un organisme quelconque.

Les études récentes que j'ai faites sur la *Spirochæta calligyra*, espèce qui occupe une place intermédiaire entre le spirochète pâle et la *Spirochæta refringens*, que l'on trouve dans les lésions non syphilitiques des régions génitales humaines, font également ressortir la difficulté qu'il y a à reconnaître un spirochète qui ressemble au spirochète pâle dans des cultures provenant directement de tissus syphilitiques humains. La *Spirochæta calligyra* se développe beaucoup plus facilement dans plusieurs milieux (y compris le sérum de cheval et le sérum agar sans tissu frais) et peut facilement être pris pour le spirochète pâle. La *Spirochæta calligyra* se développe comme le spirochète pâle et ne produit aucune odeur ni altération de milieu. Il s'en distingue par l'absence de pathogénicité et parce qu'il apparaît dans des lésions non spécifiques, et aussi parce qu'il est un peu plus gros. Les spirochètes non pathogéniques qui se cultivent facilement dans le sérum agar ordinaire ou un sérum coagulé appartiennent à ce groupe, bien qu'il soit possible que le spirochète pâle se développe symbiotiquement en même temps que le calligyrum. Dans un cas de ce genre, la culture pourrait être pathogénique mais non pas pure. C'est pour cette raison que je considère comme absolument essentiel pour obtenir une série pure de spirochètes pâles, que l'on se serve pour les cultures de matériaux purs tels que le syphilome des testicules de lapin. Dans une culture où le spirochète pâle se trouve associé au *calligyrum*, *refringens*, *microdentium* ou *mucosum*, il est facile d'éliminer le spirochète pâle des générations culturelles futures en supprimant l'addition de tissu frais et en négligeant les précautions anaérobiques rigoureuses, mais on ne saurait accomplir l'inverse.

Certains investigateurs ont appuyé sur la propriété antigénique des spirochètes cultivés par rapport à la réaction de Wassermann, mais il faut éviter de confondre cette réaction avec celle d'un véritable anticorps-antigène. Une émulsion de testicule de lapin riche en spirochète pâle ou une culture pure de spirochète pâle n'est pas capable de fixer le complément avec la plupart des sérums provenant de cas de début de syphilis, bien qu'ils donnent une réaction positive plus fréquemment dans les cas latents ou tertiaires. Dans les cas latents ou tertiaires, la réaction, selon moi, est spécifique, et peut être nettement séparée de la réaction de

Wassermann par des séries parallèles d'essais. Les résultats sont différents lorsqu'on emploie un extrait alcoolique de spirochète pâle contenant du tissu ou des cultures, puisqu'ils sont dus aux éléments lipoides du tissu aussi bien que de la masse culturale. Dans ces conditions, on peut les comparer à la réaction de Wassermann.

Les sérums de lapins ayant été immunisés fortement par des cultures pures de spirochète pâle contiennent des agglutinines spécifiques et des anticorps capables de fixer le complément (Noguchi, Kolmer, Williams et Lanbaugh, Nakano), tandis que la peau des animaux immunisés révèle un état de sensibilité exagérée à l'extrait de spirochète pâle (Noguchi). Mais la peau de lapins ayant une orchite syphilitique active ne réagit pas nettement. Neisser, Jackson, Nobl et d'autres ont démontré qu'une sensibilité exagérée de la peau se produisait dans la syphilis, et j'en ai rendu la constatation plus constante par l'emploi d'un extrait de plusieurs séries de cultures pures de spirochètes pâles. L'application en clinique de ce procédé (la réaction cutanée à la luétine) a été confirmée par Cohen, Orleman-Robinson, Wolfsohn, Nobl et Fluss, Kämmerer, Rytina, Gradwohl, Simpson, Fagliuoli et Fisichella, Geber, Bellantoni, Schmitter, Foster, Kaliski, Baermann et Heinemann et Nakano. D'autre part, Schereschewsky, en se servant de sa culture impure, n'a obtenu que des résultats imprécis.

De la courte revue ci-dessus, dans laquelle les détails techniques ont été omis à dessein, on peut déduire ce qui suit :

A n'en pas douter, la culture du spirochète pâle est chose accomplie, mais les méthodes employées jusqu'à présent donnent toujours des résultats inconstants. Lorsqu'on obtient des cultures impures de n'importe quel matériel syphilitique humain, la preuve qu'il y a des spirochètes pâles est tirée de l'inoculation animale positive ; mais, lorsqu'il n'existe pas de pouvoir pathogénique, il est impossible d'affirmer la présence de ces spirochètes. Même lorsque les cultures sont tirées de source humaine, on ne peut pas prouver que les cultures de spirochètes soient des cultures pures de spirochètes pâles, parce qu'il peut y avoir d'autres spirochètes semblables au point de vue morphologique. Ce que l'on peut dire de positif, c'est que les cultures pures de spirochètes pâles n'occasionnent pas de putréfaction, et que toute culture contenant des spirochètes semblables aux spiro-

chètes pâles, produisant une odeur et pourtant pathogéniques, doit être considérée comme impure. S'il y a putréfaction et que la culture soit non pathogénique, il n'y a aucun moyen d'établir que c'est une culture de spirochète pâle.

De ce qui précède, je déduis ce qui suit :

La première preuve formelle de la culture pure de spirochète pâle a été apportée par moi en 1911. Pour y arriver, j'ai employé une méthode anaérobie spéciale. Les investigateurs ultérieurs ont obtenu des spirochètes pâles pathogéniques par des méthodes de culture plus simples, mais, d'après moi, ils n'ont pas prouvé d'une manière certaine l'exclusion d'espèces saprophytiques alliées.

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Figure 1. Ma méthode pour obtenir la culture pure du *Treponema pallidum* dans le milieu liquide. Les tubes supérieurs contiennent le milieu solide, et les tubes inférieurs, plus larges, le milieu liquide. Dans les deux séries les cultures sont en évolution.

Figure 2. Différents spirochètes en culture pure.

1. *Treponema pallidum*.

7. *S. refringens* (pas de tissu).

2. *T. pallidum*.

8. *Treponema microdentium* (pas de tissu).

3. *T. pertenue*.

9. *T. microdentium*.

4. *T. calligyrum*.

10. *T. mucosum*.

5. *T. macrodentium*.

6. *Spirochæta refringens* (pas de tissu).

11. *Spirochæta phagedenis*.

Figure 3. Cultures pures de différents spirochètes en milieu solide.

Figure 4. *Pallidum*, type gros.—Culture pure.

Figure 5. *Pallidum*, type mince.—Culture pure.

Figure 6. Culture pure. — *Pallidum*.

Figure 7. Division longitudinale d'un tréponème.

Figure 8. Culture pure. — *Pallidum*.

Figure 9. *Pallidum*, type moyen.

Figure 10. *Pallidum*.

Figure 11. Culture pure. — *Pallidum*.

Figure 12. Tréponème pâle du tissu.

Figure 13. Formes involuées du tréponème pâle en culture peu favorable. On y trouve des corpuscules ronds ou ovales qui ressemblent à des grains chromatoides.

Figure 14. Formes granulées.

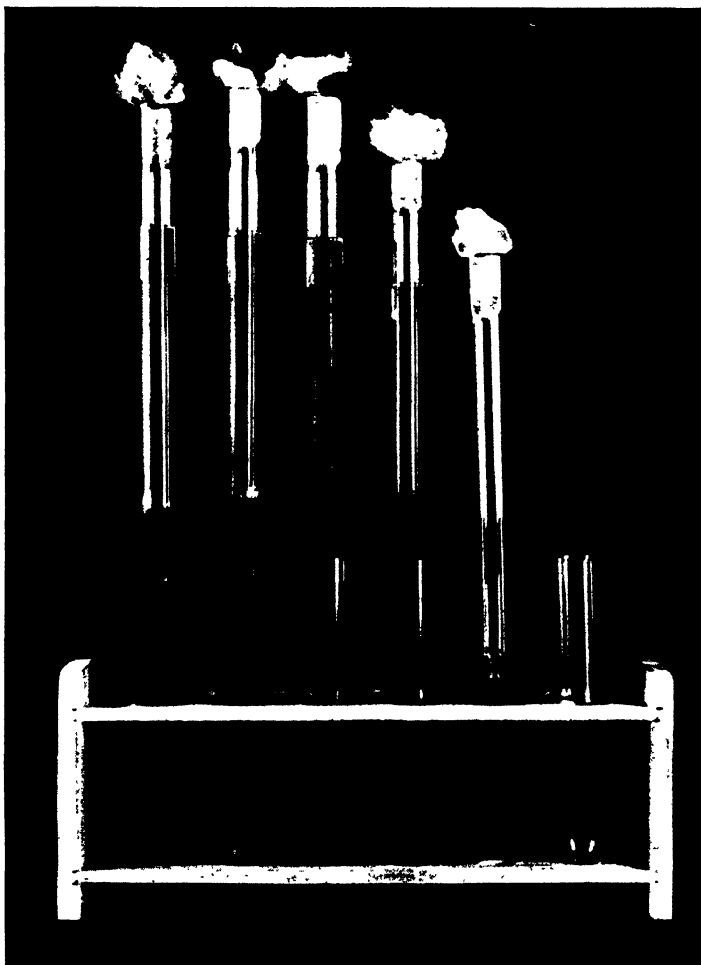


FIGURE I.

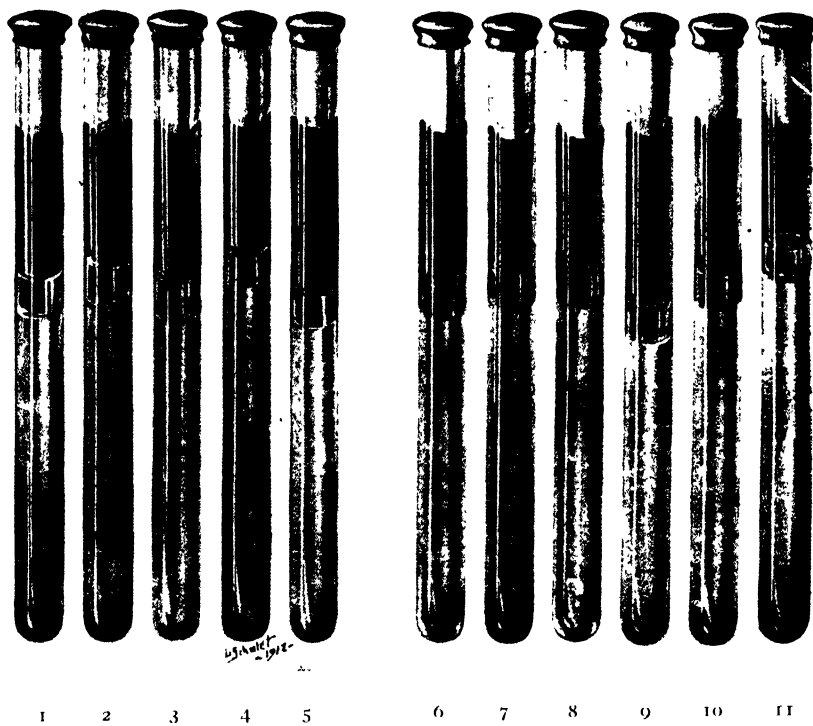


FIGURE 2.



FIGURE 3.

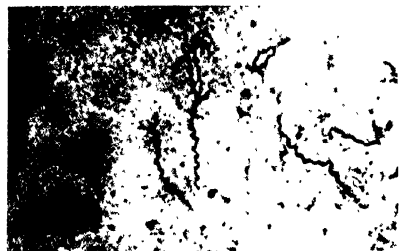


FIGURE 4.

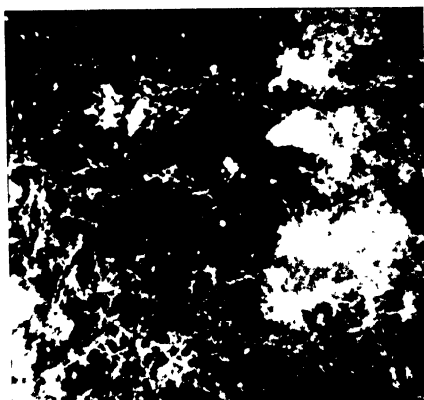


FIGURE 5.



FIGURE 6.



FIGURE 7.

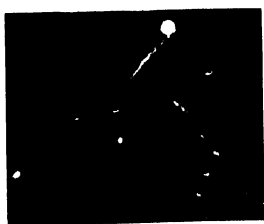


FIGURE 8.

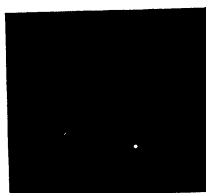


FIGURE 9.



FIGURE 10.



FIGURE 11.



FIGURE 12.



FIGURE 13.

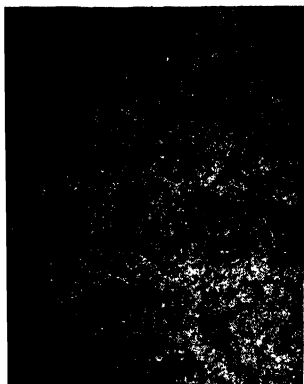


FIGURE 14.



FIGURE 16.

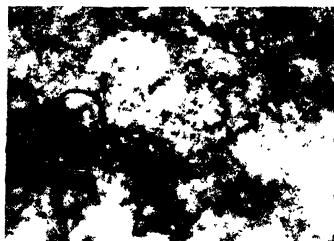


FIGURE 15.



FIGURE 17.



FIGURE 18.

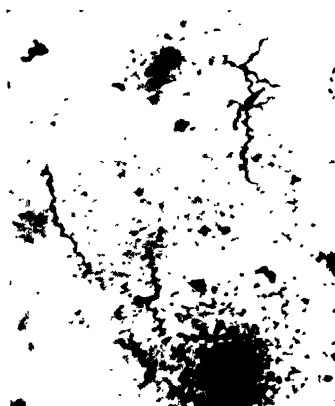


FIGURE 19.

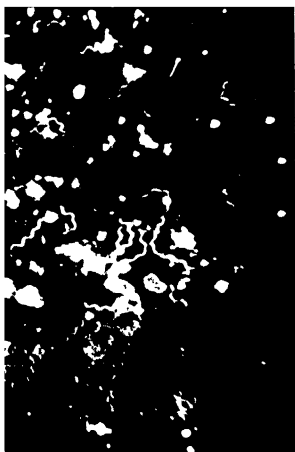


FIGURE 20.



FIGURE 21.



FIGURE 22.



FIGURE 23.



FIGURE 24.



FIGURE 25.



FIGURE 26.



FIGURE 27.

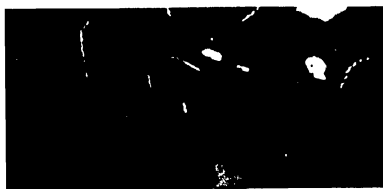


FIGURE 28.



FIGURE 29.

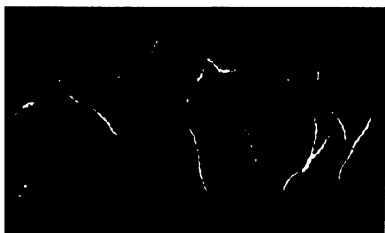


FIGURE 30.

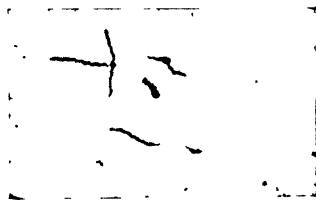


FIGURE 31.

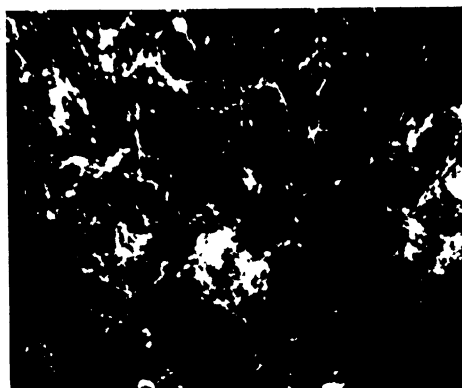


FIGURE 32.

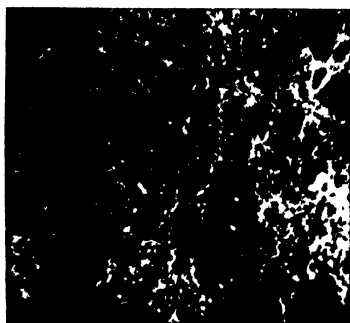


FIGURE 33.

Figure 15. Enkystement du tréponème pâle.

Figure 16. Formes granulaires. — Granules minuscules trouvés dans une culture défavorable.

Figure 17. Formes granulées.

Figure 18. Culture pure en milieu liquide. *Treponema pertenue*.

Figure 19. *Calligyrum*.

Figure 20. *Calligyrum*. — Culture pure.

Figure 21. *Calligyrum*.

Figure 22. *Macrodentium*. — Culture pure jeune.

Figure 23. *Macrodentium*. — Culture pure plus âgée.

Figure 24. *Calligyrum*.

Figure 25. *Refringens*. — Culture pure.

Figure 26. *Refringens*. — Culture pure.

Figure 27. *Microdentium*. — Culture pure en milieu liquide.

Figure 28. *Microdentium*. — Culture pure.

Figure 29. *Microdentium*. — Culture pure.

Figure 30. *Microdentium*. — Culture pure en milieu liquide.

Figure 31. *Microdentium*. — Culture pure.

Figure 32. *Treponema mucosum*. — Culture pure en milieu liquide.

Figure 33. *Treponema mucosum*. — Culture pure.

PARALYSIE GÉNÉRALE ET SYPHILIS.

LA DÉCOUVERTE DU TRÉPONÈME PÂLE DANS LE CERVEAU DE PARALYTIQUES GÉNÉRAUX. LA TRANSMISSION DU TRÉPONÈME PÂLE DU CERVEAU DE PARALYTIQUES GÉNÉRAUX AU LAPIN ET LA PRODUCTION EXPÉRIMENTALE DE L'ENCÉPHALITE SYPHILITIQUE DIFFUSE CHEZ LES ANIMAUX.

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Dans le présent article, je ne chercherai pas à traiter toute la question de la paralysie générale, mais je me bornerai à la phase du sujet qui, depuis moins d'un an, a pris un nouvel aspect.

Grâce aux investigations exactes et laborieuses de Bayle, Calmeil, Esmarch et Jessen, Kjelberg, Fournier, Mendel, Nageotte, Binswanger, Marie, Nissl, Guillaïn, Kraepelin, Alzheimer, Nonne, Krafft-Ebing, Westphal, Obersteiner, Erb, Leredde, Jolly, Régis, Wollenberg et bien d'autres encore, on est arrivé à reconnaître en la paralysie générale une affection déterminée du système nerveux central, alliée étiologiquement à la syphilis. La symptomatologie et l'anatomie pathologique de la paralysie générale ont été si bien établies et sont si connues qu'il n'est point besoin d'en parler ici. Nous savons tous que la paralysie générale se termine invariablement par la mort, que cette maladie attaque presque quatre fois plus d'hommes que de femmes, qu'elle commence dans la force de l'âge, que ses victimes comprennent de 15 à 30 pour 100 de tous ceux internés dans les asiles d'aliénés. Nous connaissons les différentes théories de la pathogénie de la paralysie générale et du tabes dorsal que Möbius, Strümpell, Fournier, Erb, Nageotte, Binswanger, Marie, Krafft-Ebing, Nissl, Kraepelin, Nonne et leurs élèves ont soutenues si énergiquement pendant les cinquante dernières années, sans parvenir à une solution satisfaisante du problème.

Bien que les statistiques des différents cliniciens prouvent que la paralysie générale se développe chez les personnes ayant eu la syphilis, il n'existait pas de preuve concluante que la paralysie est une véritable affection syphilitique. Les découvertes cytologiques, biochimiques et sérologiques (Ravaut et Sicard, Nonne et Apelt, Wassermann et Platt) ont, toutefois, rendu le rapport étroit entre la syphilis et la soi-disant parasyphilis plus probable que jamais, sans le rendre concluant, parce que la démonstration de l'agent causatif de la syphilis n'avait pas encore été faite. Nous nous trouvions donc en face d'une situation paradoxale, à savoir, que dans la parasyphilis certains phénomènes indicatifs de processus syphilitiques actifs se produisaient sans que la présence de l'organisme syphilitique fût prouvée dans l'organe atteint. Les observations d'Alzheimer, Nonne, Fournier, Krafft-Ebing et autres sur la forme juvénile de la paralysie générale et sa parenté étroite avec l'hérédosyphilis, ainsi que l'existence simultanée de lésions parétiques et syphilitiques dans certains cas rapportés par Zambaco, Westphal, L. Meyer, Binswanger, Hübner, Straussler et autres indiquaient fortement la nature syphilitique de ces affections. Les observations de Krafft-Ebing dans lesquelles il a constaté que des paralytiques résistaient à l'infection syphilitique, sont importantes sous ce rapport, puisqu'elles rattachent les processus pathologiques de la paralysie générale à ceux de la syphilis active.

On voit donc que la solution de la question épineuse du rapport de la syphilis avec la parasyphilis dépendait uniquement de la découverte de spirochètes pâles dans les lésions. Il a été consacré à cette fin bien du temps et du travail par différents investigateurs depuis la découverte mémorable de Schaudinn et Hoffmann, mais sans succès.

Malgré ces insuccès, décourageants par eux-mêmes, j'ai été amené, par l'observation que le spirochète pâle prend quelquefois une forme granulaire en culture, à étudier à nouveau des coupes de cerveaux de paralytiques colorés pour la recherche du spirochète pâle. La recherche a été très complète, car j'étais décidé à étudier toutes sortes de parcelles granulaires qui pouvaient être identifiées avec les formes granulaires du spirochète pâle, telles qu'on les trouve en culture. Après avoir étudié 69 préparations sur 70, j'aperçus

un spirochète, précisément au moment où j'allais abandonner la recherche, puisque je ne pouvais décider si les granules contenus dans les préparations étaient des formes granulaires du spirochète pâle ou des précipités. Un seul spirochète typique sur 70 préparations suffit, toutefois, à m'obliger à revoir toutes les coupes un grand nombre de fois, et cela a eu pour résultat de démontrer la présence de spirochètes pâles typiques dans 12 cas sur 70. Ces résultats ont été publiés avec M. J. W. Moore au mois de Février 1913.

Immédiatement après, nous avons étudié 130 autres cerveaux paralytiques; dans 36 cas de cette série, des spirochètes pâles ont été mis en évidence, ce qui fait environ 25 p. 100 de résultats positifs, en calculant les deux séries ensemble (48 sur 200 cas). Dans 6 cas, on a employé l'ultra-microscope avec du cerveau frais, et dans l'un de ces 6 cas, la présence de spirochètes pâles a été démontrée.

Ces résultats furent bientôt confirmés par d'autres. C'est ainsi que Marinesco et Minea ont rapporté un résultat positif sur 26 cas, par la méthode à l'argent; puis Marie, Levaditi et Bankowski ont rapporté trois résultats positifs sur 14 cas par l'imprégnation à l'argent; tous positifs dans 6 cas, morts pendant une attaque, au moyen de la méthode de Burri, ainsi qu'à l'ultra-microscope; Forster et Tomaszewski obtinrent 2 résultats positifs sur 4 cas, dans lesquels les matériaux furent tirés des malades au moyen de la ponction Neisser-Pollak et examinés à l'ultra-microscope. Forster et Tomaszewski ont observé des mouvements actifs de cet organisme.

On peut dire brièvement que le rapport entre le spirochète pâle et les éléments de tissus des cerveaux affectés est une spirochétose diffuse de tout le cerveau, affectant surtout les couches corticales. Malgré une infiltration cellulaire diffuse, l'on n'a pas encore démontré la présence de spirochètes pâles dans la pie-mère. On voit souvent que les cellules nerveuses contiennent un ou plusieurs spirochètes, tandis que la paroi des vaisseaux sanguins reste intacte dans la plupart des cas. La présence du spirochète pâle dans le parenchyme explique une grande partie des altérations histopathologiques et les symptômes observés dans le cours de la maladie. La nouvelle formation de capillaires avec augmentation des éléments gliomes de soutien et atrophie des éléments nerveux, ainsi que l'infiltration des parois vasculaires, doivent être attribuées à l'activité des spirochètes.

Les propriétés biologiques des spirochètes pâles constatés dans la paralysie générale sont d'un intérêt considérable. Les auteurs qui soutiennent la théorie syphilitique de la paralysie générale et du tabes s'appuient, pour la plupart, sur le caractère bénin de l'infection pendant les phases primaire et secondaire. Ils sont disposés à penser que l'organisme qui affectera plus tard le système nerveux central a, en quelque sorte, une affinité spécifique pour ce dernier. Les fameux cas rapportés par Brosius, dans lesquels plusieurs souffleurs de verre infectés à la même source ont été atteints plus tard soit de paralysie, de taboparalysie ou de syphilis cérébro-spinale sont connus. Qu'il y ait un *virus nerveux* parmi les spirochètes pâles est important au point de vue pratique, mais jusqu'à ce jour il n'a été fait aucune investigation exacte sur ce point.

Afin d'établir, premièrement, le caractère infectieux, et ensuite les propriétés des spirochètes trouvés dans le cerveau dans la paralysie générale, j'ai entrepris une série d'expériences pour transmettre le spirochète pâle directement d'un cerveau de paralytique à un lapin. Trente-six lapins ont été inoculés avec les émulsions de six spécimens différents de cerveaux de paralytiques à l'état frais.

On a remarqué chez deux des six lapins inoculés avec le matériel tiré de l'un des cas, au bout de quatre-vingt-dix-sept jours pour l'un et de cent deux jours pour l'autre, une induration, petite, mais typique, dans le parenchyme testiculaire et la peau scrotale. Dans le premier cas, les spirochètes pâles étaient très peu nombreux, tandis que dans le deuxième ils étaient présents en abondance. Le développement des lésions fut exceptionnellement lent lorsqu'on le compare aux transmissions faites de chancres ordinaires ou lésions secondaires chez cet animal; dans ce dernier cas, les lésions apparaissent généralement dans un intervalle de quatre à six semaines, rarement deux mois. Le deuxième groupe de cette série présenta également d'assez petites lésions, indurées légèrement seulement, chez l'un des quatre lapins inoculés aux testicules; la période d'incubation fut, encore une fois, de près de trois mois. Mes expériences ont été limitées à un petit nombre de spécimens de cerveaux (six), mais elles ont néanmoins démontré le caractère in-

fectieux des spirochètes présents dans un cerveau paralytique, et établi que leur virulence était faible pour le lapin.¹

Nichols et Hough ont également provoqué la kératite syphilitique chez le lapin au moyen de l'inoculation intratesticulaire de l'émulsion d'un cerveau de paralytique. Toutefois, dans leur cas, ils n'ont pas réussi à trouver des spirochètes pâles dans la lésion, mais la lésion de kératite a été transmise à une série ultérieure de lapins. Graves a rapporté qu'il avait réussi à provoquer non seulement la kératite, mais encore des papules de la peau dans le prépuce de lapins, en leur inoculant du sang de paralytiques. Les données disponibles jusqu'ici ne nous permettent pas de tirer aucune conclusion en ce qui concerne l'existence d'une espèce qui soit spécifique pour le système nerveux central, mais elles établissent le caractère infectieux de l'organisme que l'on trouve dans la paralysie générale.

La cause du long intervalle entre l'époque d'infection syphilitique primaire et le développement des symptômes parétiques—en moyenne de 8 à 12 ans—est toujours inconnue. Entre le caractère latent et la pathogénésie de la paralysie, il peut y avoir un rapport déterminé. Pourquoi la syphilis cérébrale ou cérébro-spinale apparaît-elle beaucoup plus tôt que la soi-disant parasyphilis? Je ne connais pas de réponse satisfaisante à cette question. A ce sujet, je rapporterai brièvement certains résultats expérimentaux obtenus récemment. Le système nerveux central des singes et des en ce qui concerne l'existence d'une espèce qui soit spécifique pour le virus est introduit directement dans la substance cérébrale. La plupart des animaux restent en parfaite santé pendant une période indéfinie après l'inoculation intra-cérébrale de spirochète pâle. Il est probable que ces animaux ont besoin d'être sensibilisés avant que le spirochète pâle puisse infecter le cerveau. C'est pour cette raison que j'ai fait à ces animaux des inoculations intraveineuses répétées, de spirochète pâle mort et vivant, pendant cinq mois, et qu'ensuite après un intervalle de cinq mois j'ai fait une inoculation intra-céré-

1. Dans leur dernier article, MM. Levaditi, Marie et Bankowski mentionnaient que Landsteiner aurait réussi à transmettre la syphilis au singe par l'inoculation de matière cérébrale provenant d'un cas de paralysie générale, mais l'expérience de cet auteur n'était pas définitive.

brale d'émulsion ou l'insertion subdurale d'une parcelle minuscule de syphilome testiculaire (lapin) riche en spirochètes pâles. Douze lapins ont composé cette série d'expériences. Quatre lapins normaux (jeunes) ont été inoculés avec même matériel, le même jour, et ont servi de contrôle. Tous les animaux sont restés sains en apparence pendant deux mois, puis quelques-uns des animaux sensibilisés sont devenus stupéfiés, inactifs, émaciés, avec une spasticité définie des membres inférieurs, et une légère ataxie. Les réactions de la pupille ne pouvaient servir au diagnostic, car normalement les pupilles sont souvent de grandeur différente et lentes à répondre aux stimuli de la lumière. Les manifestations ont progressé constamment chez les animaux sensibilisés. Au bout de trois à cinq mois, ils ne pouvaient plus sauter. Leurs sérums, qui donnaient à l'origine des réactions de Wassermann négatives, chez quelques-uns donnaient des réactions positives. Pendant la même période, les animaux de contrôle restèrent tous actifs, à l'exception de quelques-uns d'entre eux qui accusèrent une perte de poids; aucun d'eux n'eut de réaction Wassermann de positive.

On sacrifia les animaux les uns après les autres dans un intervalle de trois à cinq mois, pour examiner les cerveaux. Il est impossible de décrire ici les résultats; je dirai simplement que, parmi les cerveaux d'animaux sensibilisés, je trouvai de la méningite exsudative non purulente diffuse trois fois, de l'atrophie unilatérale marquée des lobes frontaux une fois, de la fermeté diffuse du cerveau deux fois, et quelques minuscules plaques jaunes, d'environ 2×2 mm., dans la région temporale, une fois. L'examen à l'ultra-microscope n'a pas révélé de spirochètes pâles, mais comme les spécimens n'ont pas été examinés à fond, afin de les conserver pour des études histologiques, je n'attache pas d'importance à ce point. Les coupes colorées à l'imprégnation par l'argent montrèrent dans un cerveau de nombreux granules et quelques spirochètes bien déterminés. Dans d'autres, on n'a pas trouvé de spirochète pâle.

Les coupes de plusieurs animaux colorées au bleu de toluidine ou à l'hématoxyline-éosine révélèrent une prolifération endothéliale bien marquée et une infiltration périvasculaire ayant quelque ressemblance avec les spécimens de paralysie générale humaine. Dans quelques endroits, il y eut augmentation des cellules gliomes, tandis

que la plupart des cellules nerveuses restèrent intactes. Les cerveaux des animaux de contrôle n'ont pas accusé de changement dans les vaisseaux capillaires, quoique dans un cas l'on a observé une lymphocytose diffuse dans tout le cerveau. Les expériences ci-dessus ne sont pas sans intérêt pour l'interprétation de la pathogénésie de la parésie générale, puisqu'elles indiquent que la sensibilisation antérieure rend vulnérable à l'invasion du spirochète pâle le système nerveux central d'animaux qui y seraient sans cela réfractaires.

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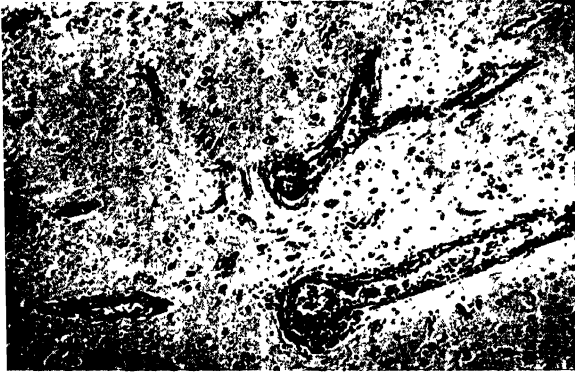


FIGURE 1.



FIGURE 2.



FIGURE 3.

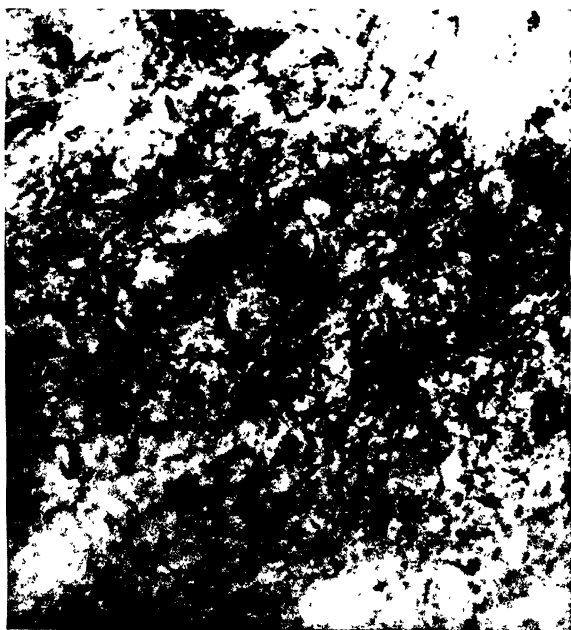


FIGURE 4.

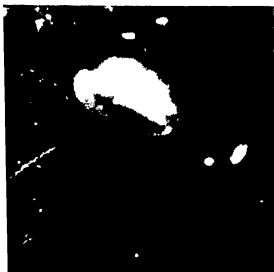


FIGURE 5.



FIGURE 6.



FIGURE 7.



FIGURE 8.

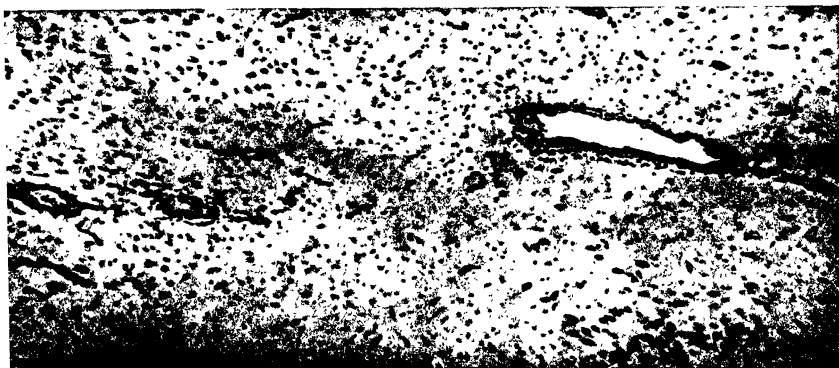


FIGURE 9.



FIGURE 10.

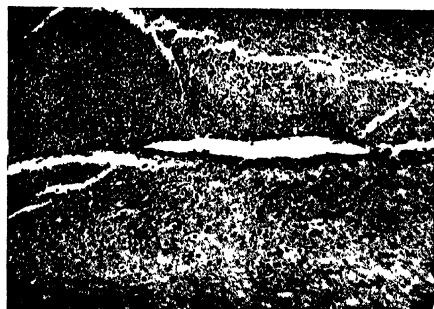


FIGURE 11.



FIGURE 12.

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Figure 1. Lésions typiques du cerveau dans un cas de paralysie générale.

Figure 2. Lésions très prononcées du cerveau dans un cas de paralysie générale.

Figure 3. Le tréponème pâle dans le cerveau d'un cas de paralysie générale.

Figure 4. Le tréponème pâle dans le cerveau d'un cas de paralysie générale mort en iclus.

Figure 5. Le tréponème pâle vivant trouvé à l'ultra-microscope dans l'émulsion préparée du cerveau d'un cas de paralysie générale.

Figure 6. Transmission au lapin dans le testicule de spirochètes pâles pris dans le cerveau d'un paralytique.

Figure 7. Spirochète trouvé dans les lésions de transmission.

Figure 8. Spirochète trouvé dans les lésions de transmission.

Figure 9. Les lésions caractéristiques du cerveau chez un lapin préalablement sensibilisé et puis inoculé du tréponème pâle subduralement.

Figure 10. Lésions vasculaires et quelques foyers d'indurations cellulaires dans les couches corticales chez un lapin préalablement sensibilisé et puis infecté sous la dure-mère du cerveau.

Figure 11. Épendymite chez un lapin syphilitique.

Figure 12. Les tréponèmes pâles dans les couches corticales d'un lapin préalablement sensibilisé et puis inoculé dans le cerveau.

THE RELATION OF THE RATE OF ABSORPTION OF ADRENALIN TO ITS GLYCOSURIC AND DIURETIC EFFECTS.*

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INTRODUCTION.

Deep or intramuscular injections have been practised by physicians for many years. This was not done, however, with the expectation that it would favor absorption; on the contrary, some writers (1) state especially, "that the absorption from the intramuscular tissue with its fewer lymphatics is much slower than from the subcutaneous tissues where the lymphatics are abundant." The intramuscular injection was recommended because of the assumption that "deep injections give rise less often to the formation of abscesses." About nine years ago Meltzer and Auer (2) showed experimentally for the first time that the effects of active drugs appear after intramuscular injections earlier and with greater intensity than after subcutaneous injections. The experiments were made with adrenalin, curare, fluorescëin, and morphin. Doses of curare, for instance, which practically had no effect in subcutaneous injections, brought on paralysis and death when injected intramuscularly. In recent experiments by the same authors (3) in which the previous results regarding the blood pressure action of adrenalin were especially studied and confirmed, potassium cyanide was added to the list of substances which act more profoundly by intramuscular injection. They also found that acute anaphylactic shock can be brought on in highly sensitized guinea pigs by injecting one cubic centimeter of horse serum into the lumbar muscles; whereas such a prompt result is never obtained by a subcutaneous

* Received for publication, June 6, 1913.

injection. Eggleston and Hatcher (4) studied recently the seat of the emetic action of apomorphin, and found that for intramuscular injections the minimum dose of this drug is half that which produces a similar effect upon subcutaneous injection. Of practical importance are the experiments of Morgenroth and Levy (5) on the absorption of diphtheria antitoxin. They found that the intramuscular injection of a small dose of antitoxin, together with a fatal dose of toxin, prevented even induration at the seat of injection, while a much larger dose of the antitoxin administered subcutaneously (again together with a fatal dose of toxin) was incapable of preventing the early death of the animals. It seems therefore to be now a well established fact that substances, whether crystalloidal or colloidal, producing definite effects upon the animal body, manifest these effects earlier and with greater intensity when administered by intramuscular than by subcutaneous injection.

The earlier experiments of Meltzer and Auer with adrenalin dealt with three of its known effects: (a) rise of blood pressure; (b) dilatation of the pupil on the side in which the superior cervical ganglion has been removed; and (c) manifest muscular weakness which follows the injection of larger doses of the drug. These three different manifestations appeared in an unmistakable manner quite rapidly after an intramuscular injection of adrenalin, while after subcutaneous injection of a similar dose of that drug, either no effect at all followed, or the effect was comparatively insignificant and appeared late. Adrenalin, however, is capable of producing another quite important effect upon the animal body, namely, the production of glycosuria. Since the discovery of adrenalin glycosuria by Blum (6), the subject has been studied by many investigators. The influence of the place of injection upon the intensity of the glycosuria has also been variously investigated. For dogs it was stated by Herter and Wakeman (7) that intravenous injections give better effects than subcutaneous, and that intraperitoneal injections give the best effects. For the rabbit the glycosuric effects of subcutaneous injections were compared with those of intravenous injections by Ritzmann (8). According to him the glycosuric effect of adrenalin is similar to that of the same drug upon blood pressure; in both cases intravenous injection of the

drug is more effective than subcutaneous. However, among the numerous recent communications on adrenalin glycosuria no mention is made of the glycosuria after intramuscular injection of an adrenal preparation.

The foregoing brief review brings out the following points. It is now quite safely established that active crystalloidal and colloidal substances are pharmacologically more effective when administered by intramuscular than by subcutaneous injections. This fact has been established especially for the several effects produced by adrenalin when injected into the animal body. The glycosuric action of drugs, however, has not been studied as yet by the method of intramuscular injection. This gap we have tried to fill in the present investigation. We have studied the effect of intramuscular as compared with the subcutaneous injection of adrenalin upon the production of glycosuria.

EXPERIMENTAL DATA.

Method.—The experiments were performed on rabbits. There were forty-nine experiments with two rabbits for each, one of which received the adrenalin intramuscularly and the other subcutaneously. The intramuscular injections were given in the lumbar muscles since, as Auer and Meltzer (9) have shown for rabbits, the absorption is better from these muscles than from any other mass of muscular tissue; the subcutaneous injections were made in one of the lower quadrants of the abdomen. The adrenalin¹ was employed in the usual solution of 1:1,000. Many of the rabbits were not fed for twenty-four hours previous to the experiment. Most of the animals received, immediately before the injection of adrenalin, 100 or 150 cubic centimeters of water through a stomach tube. None of the rabbits were fed during the time of observation after the administration of adrenalin, which usually was twenty-four hours. During the observation the animals were kept in metabolism cages, the bladder being emptied at the beginning and at the end of the period of observation. No experiment was considered as finished until the urine was sugar-free. The urines were always preserved with toluene. The analysis for sugar was made by the Pavy-

¹ The adrenalin employed was prepared by Parke, Davis and Company.

Ramsden method,² the reduction being calculated as dextrose. Although rabbit urine always contains some substance which slightly reduces the ammoniacal copper solution, the inaccuracy resulting from it is too insignificant to impair the results in any degree, since our object was not to ascertain the absolute amount of reducing substances present in the urine, but merely a comparison of the amounts of these substances brought about by two different methods of administration of adrenalin.

Results.—As mentioned before, ninety-eight rabbits were injected with adrenalin, one half intramuscularly and the other half subcutaneously. The injections were made in pairs; that is, in each experiment two rabbits were employed at the same time and under

TABLE I.

Adrenalin, 1.0 Cubic Centimeter; Water per Os, 100 Cubic Centimeters.

All Animals Fasted Twenty-Four Hours Previous to Injection.

Intramuscular injection.					Subcutaneous injection.				
No. of animal.	Weight in gm.	Urine in c.c.	Dextrose in urine in gm.	Hours.	No. of animal.	Weight in gm.	Urine in c.c.	Dextrose in urine in gm.	Hours.
A 109	1,430	68	1.48	24	A 110	1,440	160	1.43	24
A 111	1,480	174	1.10	24	A 112	1,555	160	0.34	24
A 113	2,085	143	0.35	24	A 114	2,110	131	1.38	24
A 115	2,310	104	0.13	24	A 116	2,325	>167	1.18	24
A 117	—	—	—	Died	A 118	1,435	109	2.02	24
A 119	1,840	101	2.85	24	A 120	1,520	88	1.77	24
A 121	1,425	53	0.00	24					
A 123	1,085	58	0.00	24	A 124	1,020	129	0.64	24
A 125	1,390	98	0.83	24	A 126	1,340	147	0.65	24
A 127	1,290	137	1.09	24	A 128	1,225	>179	>3.07	24
A 129	1,430	120	0.76	24	A 130	1,350	139	1.98	24
Average		106	0.86		Average		141	1.45	

the same conditions, each being injected with a like quantity of adrenalin, the one receiving it intramuscularly and the other subcutaneously. The two sets of results are therefore comparable. Different doses of adrenalin were tested, one cubic centimeter of a 1:1,000 solution being the highest and 0.3 about the lowest dose which was systematically tested. The forty-nine experiments consist therefore actually of several series of experiments, which run as fol-

² Vernon, H. M., *Jour. Physiol.*, 1902, xxviii, 164.

lows. Ten pairs of rabbits received 1 cubic centimeter per animal, seven pairs received 0.7 of a cubic centimeter, ten pairs received 0.5, eight pairs received about 0.4, and eight pairs received 0.3 of a cubic

TABLE II.

*Adrenalin, 0.7 to 0.8 of a Cubic Centimeter; Water per Os, 100 Cubic Centimeters.
All Animals Fasted Twenty-Four Hours Previous to Injection.*

Intramuscular injection.					Subcutaneous injection.				
No. of animal.	Weight in gm.	Urine in c.c.	Dextrose in urine in gm.	Hours.	No. of animal.	Weight in gm.	Urine in c.c.	Dextrose in urine in gm.	Hours.
A 73	1,820	100	1.15	24	A 74	2,060	132	0.99	24
A 75	2,220	149	1.53	24	A 76	2,410	102	1.13	30
A 77	1,070	103	1.00	48	A 78	1,160	96	0.75	48
A 79	1,530	90	0.77	24	A 80	1,550	121	1.76	24
A 85	1,920	111	0.00	24	A 82	1,360	124	1.02	24
A 89	1,160	48	0.00	24	A 84	1,580	175	1.10	24
Average		100	0.74		Average		125	1.13	

centimeter. In addition there were six pairs in which the injected dose varied from 0.7 to 0.8 of a cubic centimeter per animal. The results are given in detail in tables I to VI, each table containing

TABLE III.

*Adrenalin, 0.7 of a Cubic Centimeter; Water per Os, 150 Cubic Centimeters.
Animals Not Previously Fasted.*

Intramuscular injection.					Subcutaneous injection.				
No. of animal.	Weight in gm.	Urine in c.c.	Dextrose in urine in gm.	Hours.	No. of animal.	Weight in gm.	Urine in c.c.	Dextrose in urine in gm.	Hours.
A 29	2,170	176	1.05	24	A 30	2,140	145	1.33	24
A 31	1,750	175	0.36	49	A 32	2,170	179	1.74	49
A 33	1,595	171	0.83	48	A 34	1,860	203	1.80	48
A 35	1,550	49	0.00	24	A 36	1,500	206	3.08	24
			(Trace?)						
A 37	2,170	222	2.69	48	A 40	1,990	276	1.96	48
A 41	1,820	176	0.21	48	A 42	1,720	228+	0.00	48
								(Trace?)	
A 43	2,920	282	2.23	48	A 44	2,890	265	3.01	48
Average		179	1.06		Average		215	1.86	

the data obtained with a given dose of adrenalin, and all six tables are summarized in table VII. In every case the results of two injec-

tions which are placed opposite one another in the tables were done at the same time.

TABLE IV.

Adrenalin, 0.5 of a Cubic Centimeter; Water per Os, 100 to 150 Cubic Centimeters.

Intramuscular injection.					Subcutaneous injection.				
No. of animal.	Weight in gm.	Urine in c.c.	Dextrose in urine in gm.	Hours.	No. of animal.	Weight in gm.	Urine in c.c.	Dextrose in urine in gm.	Hours.
A 45 ³	1,610	196	1.44	24	A 46 ³	1,540	198	3.62	24
A 47 ³	1,820	74	0.00	24	A 48 ³	1,640	114	0.27	24
A 89 ⁴	1,300	151	0.34	24	A 90 ⁴	1,310	181	0.75	24
A 91 ⁴	1,400	154	0.38	24	A 92 ⁴	1,410	160	1.01	24
A 93 ⁴	1,750	87	1.16	24	A 94 ⁴	1,800	193	0.33	24
A 95 ⁴	2,185	63	0.34	24	A 96 ⁴	2,290	184	0.45	24
A 105 ⁴	1,500	157	0.26	24	A 106 ⁴	1,555	165	1.09	24
A 107 ⁴	1,630	150	0.80	24	A 108 ⁴	1,675	213	2.57	24
A 101 ⁴	1,175	89	0.00	24	A 102 ⁴	1,300	165	0.00	24
A 103 ⁴	1,415	103	1.44	24	A 104 ⁴	1,550	124	1.02	24
Average		122	0.62		Average		170	1.11	

On glancing through the various tables, we are confronted, in the first place, with a discouraging element; the method does not lend itself to the obtaining of uniform quantitative results. Under ap-

TABLE V.

*Adrenalin, 0.4 of a Cubic Centimeter; Water per Os, 100 Cubic Centimeters.
Animals Not Previously Fasted.*

Intramuscular injection.					Subcutaneous injection.				
No. of animal.	Weight in gm.	Urine in c.c.	Dextrose in urine in gm.	Hours.	No. of animal.	Weight in gm.	Urine in c.c.	Dextrose in urine in gm.	Hours.
A 49 ⁵	2,070	89	0.00	24	A 50 ⁵	1,970	136	0.88	24
A 53	1,630	148	0.87	24	A 54	1,395	159	0.00	24
A 55	1,750	104	0.62	24	A 56	1,670	182	0.43	24
A 63	1,500	101	0.48	24	A 64	1,380	141	2.30	24
A 65	1,670	103	0.00	24	A 66	1,510	102	0.84	24
A 67	1,820	171	2.46	24	A 68	1,720	154	1.44	24
A 69 ⁶	1,530	117	0.00	24	A 70	1,360	102	1.73	24
A 71 ⁷	1,250	87	0.24	24	A 72 ⁸	1,220	146	0.13	24
Average		115	0.58		Average		140	0.97	

³ Not previously fasted. Received 150 c.c. of water *per os*.

⁴ Fasted twenty-four hours before experiment. Received 100 c.c. of water *per os*.

⁵ Received 150 c.c. of water *per os*.

⁶ Adrenalin, 0.45 c.c.

⁷ Adrenalin, 0.38 c.c.

⁸ Adrenalin, 0.37 c.c.

TABLE VI.

Adrenalin, 0.3 of a Cubic Centimeter; Water per Os, 100 Cubic Centimeters.

Intramuscular injection.					Subcutaneous injection.				
No. of animal.	Weight in gm.	Urine in c.c.	Dextrose in urine in gm.	Hours.	No. of animal.	Weight in gm.	Urine in c.c.	Dextrose in urine in gm.	Hours.
A 51 ⁹	1,270	112	0.00	24	A 52 ⁹	1,080	155	1.88	24
A 57 ⁹	1,650	87	0.43	24	A 58 ⁹	1,600	99	0.79	24
A 59 ⁹	1,820	69	0.16	24	A 60 ⁹	1,700	89	0.00	24
A 61 ⁹	2,270	122	2.32	24	A 62 ⁹	1,940	94	0.20	24
A 97 ¹⁰	1,400	72	0.00	24	A 98 ¹⁰	1,505	149	0.54	24
A 99 ¹⁰	1,525	86	0.00	24	A 100 ¹⁰	1,525	162	0.11	24
A 131 ¹⁰	1,490	99	0.17	24	A 132 ¹⁰	1,480	118	0.12	24
A 133 ¹⁰	2,070	161	0.29	24	A 134 ¹⁰	2,050	106	0.27	24
Average		101	0.42		Average		122	0.49	

TABLE VII.

Summary of Tables I to VI.

	Manner of injection.	No. in which no glycosuria occurred.	Urine in c.c.	Dextrose in urine in gm.
Table I. 1.0 c.c. adrenalin	Intramuscular	2	106	0.86
Fasted—10 pairs	Subcutaneous	0	141	1.45
Table II. 0.7—0.8 c.c. adrenalin	Intramuscular	2	100	0.74
Fasted—6 pairs	Subcutaneous	0	125	1.13
Table III. 0.7 c.c. adrenalin	Intramuscular	1	179	1.05
Not fasted—7 pairs	Subcutaneous	1	215	1.85
Tables II and III. 0.7—0.8 c.c. adrenalin 13 pairs	Intramuscular	3	142	0.91
	Subcutaneous	1	172	1.52
Table IV. 0.5 c.c. adrenalin	Intramuscular	2	122	0.62
2 pairs not fasted, 8 pairs fasted—10 pairs	Subcutaneous	1	170	1.11
Table V. 0.4 c.c. adrenalin	Intramuscular	3	115	0.58
Not fasted—8 pairs	Subcutaneous	1	140	0.97
Table VI. 0.3 c.c. adrenalin	Intramuscular	3	101	0.42
4 pairs fasted, 4 pairs not fasted—8 pairs	Subcutaneous	1	122	0.49
Tables I to VI. 0.3—1.0 c.c. adrenalin. All experiments—49 pairs	Intramuscular	13	120	0.73
	Subcutaneous	4	152	1.20

⁹ Not previously fasted.¹⁰ Fasted twenty-four hours previous to experiment.

parently similar conditions and by the same mode of administration a given dose of adrenalin gave rise to very variable quantities of dextrose in the urine. This held true for both modes of injection. Under these circumstances we might readily expect that in one experiment it would be an intramuscular injection of adrenalin, which would give rise to a larger quantity of dextrose in the urine, while in another experiment the larger quantity of dextrose would follow a subcutaneous injection. Evidently a small number of experiments would not permit one to draw any definite conclusions. However, the number of experiments is great enough to bring out the differences produced by the two modes of injection even under these discouraging circumstances. Hence an analysis of the various data given in the tables shows unmistakably that subcutaneous injections of adrenalin give rise more often to the appearance of large quantities of dextrose in the urine than do intramuscular injections,—a conclusion quite opposite to that which we had reason to expect. When we take, in the first place, the amount of sugar eliminated by all the forty-nine animals of each set, we find that the average amount for each animal is 1.2 grams for the subcutaneous and 0.73 of a gram for the intramuscular injections. *The glycosuria produced by intramuscular injection is less than two thirds of that produced by the same dose of adrenalin administered subcutaneously.* The difference in favor of the subcutaneous injection remains constant for every dose of adrenalin tested. But with such a small, practically minimum dose, as 0.3 of a cubic centimeter per animal (table VI), the difference in favor of the subcutaneous injection is so small as to be within the limits of experimental error.

The same holds true in general when the dose of adrenalin administered is calculated per kilo of body weight of the animal (table VIII). Here again the average amount of dextrose appearing in the urine after an intramuscular injection of adrenalin is tangibly less than that which follows a subcutaneous injection. The difference in favor of the subcutaneous injection is manifest for every dose of adrenalin used, except for doses below 0.2 of a cubic centimeter per kilo of body weight.

Another instructive fact is the number of failures occurring with both modes of administration. With each mode of administration

there were rabbits which did not react with a glycosuria after an injection of adrenalin; but out of the forty-nine intramuscular injections there were thirteen failures, while of the same number of

TABLE VIII.

Summary of Results with Dose Calculated to Body Weight.

Adrenalin in c.c. per kilo.	No. of experiments performed.	Manner of injection.	Urine in c.c.	Dextrose in urine in gm.
0.70 and over	6	Intramuscular	89	0.69
	5	Subcutaneous	141	1.67
0.50-0.69	5	Intramuscular	103	1.23
	7	Subcutaneous	132	1.32
0.40-0.49	8	Intramuscular	118	0.39
	4	Subcutaneous	183	1.43
0.35-0.39	5	Intramuscular	147	0.85
	6	Subcutaneous	186	1.13
0.30-0.34	7	Intramuscular	158	0.93
	10	Subcutaneous	149	1.49
0.25-0.29	4	Intramuscular	103	0.63
	5	Subcutaneous	150	1.07
0.20-0.24	9	Intramuscular	121	0.65
	8	Subcutaneous	169	0.87
Below 0.20	5	Intramuscular	106	0.64
	4	Subcutaneous	97	0.32

subcutaneous injections there were only four failures. Whatever the factor may be which interferes with the production of glycosuria, it is manifest that it operates more frequently when the adrenalin is administered intramuscularly than when it is administered subcutaneously, which is in harmony with the finding that the glycosuria occurring after a subcutaneous injection of adrenalin is generally greater than after an intramuscular one.

It might be supposed that the diminished averages of the urinary sugar output and the larger number of failures after intramuscular injections of adrenalin are not two separate facts, but that the lower averages after intramuscular injections are merely caused by the greater number of failures, and that the actual glycosurias, when they occur, are as severe as those, or perhaps even more severe than

those produced by subcutaneously administered adrenalin. That this is not the case may be seen from table IX, which gives the actual glycosuria for each dose. In making up the averages, the animals which did not react with glycosuria were excluded from

TABLE IX.
Summary of Actual Glycosurias.

	Manner of injection.	No. in which no glycosuria occurred.	Average excluding the cases having no glycosuria. Urine in c.c.	Average excluding the cases having no glycosuria. Dextrose in gm.
Table I. 1.0 c.c. adrenalin.....	Intramuscular	2	118	1.07
Fasted—10 pairs.....	Subcutaneous	0	141	1.45
Table II. 0.7-0.8 c.c. adrenalin.....	Intramuscular	2	111	1.11
Fasted—6 pairs.....	Subcutaneous	0	125	1.13
Table III. 0.7 c.c. adrenalin.....	Intramuscular	1	200	1.23
Not fasted—7 pairs.....	Subcutaneous	1	212	2.15
Table II and III. 0.7-0.8 c.c. adrenalin 13 pairs.....	Intramuscular	3	164	1.18
	Subcutaneous	1	169	1.65
Table IV. 0.5 c.c. adrenalin.....	Intramuscular	2	133	0.77
Not fasted—2 pairs, fasted—8 pairs.....	Subcutaneous	1	170	1.23
Table V. 0.4 c.c. adrenalin.....	Intramuscular	3	120	0.93
Not fasted—8 pairs.....	Subcutaneous	1	138	1.11
Table VI. 0.3 c.c. adrenalin.....	Intramuscular	3	108	0.67
Fasted—4 pairs, not fasted—4 pairs.....	Subcutaneous	1	126	0.56

the calculations. The proportion of the average glycosuria for the subcutaneous and intramuscular injections after this exclusion is 7.63 to 5.78 grams or 100:76. A difference in favor of the subcutaneous injection after exclusion of the failures is present also in the averages made up for each dose tested, except for the smallest dose of 0.3 of a cubic centimeter per kilo of body weight.

The experiments clearly indicate that the chances for the production of glycosuria by adrenalin are greater when it is administered subcutaneously than when given intramuscularly, and that, when glycosuria is produced, the amount of dextrose in the urine is likely to be greater after a subcutaneous injection than after an intramuscular one.

The Volume of Urine after Subcutaneous and Intramuscular Injections of Adrenalin.—All the forty-nine pairs of rabbits received, as stated above, 100, and, in some experiments, 150 cubic centimeters of water, shortly before the injection of adrenalin. Owing probably to this precaution, the diuresis was sufficient for all the rabbits; there were no failures in this respect. The largest quantity of urine passed in twenty-four hours by an individual animal was 276 cubic centimeters (subcutaneous injection), and the smallest quantity amounted to forty-nine cubic centimeters (intramuscular injection); both animals belonged to the group which received 150 cubic centimeters of water and 0.7 of a cubic centimeter of adrenalin (table III). The average quantity of urine for all animals was about 100 cubic centimeters. Here again the average volume of urine of the intramuscular was less than that of the subcutaneous animals, the proportion being 120:152, or 4:5. The proportion for the dextrose was 2:3. The difference in the quantity of urine in favor of the subcutaneous animals is manifest in each of the groups of animals receiving the same dose of adrenalin.

That the variations in the amount of dextrose in the urine were not due to the variations in the quantities of urine passed, is evident from the fact that there were, as mentioned above, among both classes of animals instances in which there was no dextrose in the urine, while some of the quantities of sugar-free urine which these animals passed were: 228, 165, 117, and 112 cubic centimeters in twenty-four hours. On the other hand, we met with instances showing fairly low volumes of urine containing relatively large quantities of dextrose; for instance, 88 cubic centimeters, 1.77 grams; 68 cubic centimeters, 1.48 grams; 87 cubic centimeters, 1.16 grams. While these figures bear witness to the existence of a considerable independence of the glycosuria from the diuresis, in our experiments a survey of all the figures shows that in a general way a certain parallelism prevails between the two phenomena, so that the larger quantities of urine are often accompanied by relatively larger quantities of dextrose, and *vice versa*.

THE EFFECT OF DISTRIBUTION OF A SUBCUTANEOUS DOSE
OF ADRENALIN.

In the foregoing experiments the fact has been brought out that in two respects the subcutaneous injection of adrenalin proved to be superior to the intramuscular; it caused a more marked diuresis and favored the appearance of dextrose in the urine. As regards the latter, the amount of dextrose was perceptibly higher, and the number of failures to react with a glycosuria was definitely smaller after subcutaneous than after intramuscular injection. These results are diametrically opposite to those previously obtained by Meltzer and Auer in testing some of the other reactions of adrenalin upon the animal body. Here the effects were unquestionably more favorable after intramuscular injections. In these experiments the favorable action was ascribed to a better absorption of adrenalin from the intramuscular tissue. Could the results of the present series of experiments mean that with regard to the effects upon diuresis and the appearance of dextrose in the urine adrenalin is better absorbed from the subcutaneous than from the muscular tissue? Such a conclusion could be accepted only on the basis of an assumption that adrenalin consists of a complex of substances; that, for instance, the action upon the blood vessels emanates from one substance which is better absorbed from the muscle tissue, and that glycosuria is produced by another component of adrenalin which is better absorbed from the subcutaneous tissue,—an assumption not readily acceptable. Or could it be that the favorable effect of the subcutaneous injections in the experiments was due to the fact that absorption from the subcutaneous tissue is less rapid? Were this the case, then any factor which favors absorption in general ought to prove an unfavorable element in the production of glycosuria by adrenalin. The last mentioned point has been tested by us in the following manner. Meltzer (10) showed some years ago that the distribution of a substance over several areas of the body increases its effectiveness. A dose of strychnin, for instance, becomes more effective when injected into several places. We have therefore tested the influence of the distribution of adrenalin over several places upon the production of glycosuria and

diuresis. This series comprised twenty rabbits, of which twelve received 0.7 of a cubic centimeter of adrenalin as a dose, six received 0.75, and two received 0.8 of a cubic centimeter. The adrenalin was given subcutaneously and each dose was distributed over four places. Each animal received 100 cubic centimeters of water previous to the injections of adrenalin. The detailed results are given in table X. Table II contains the results of

TABLE X.

Subcutaneous Distribution of Adrenalin.

Adrenalin, 0.7 to 0.8 of a Cubic Centimeter, Distributed in Four Places Subcutaneously; Water per Os, 100 Cubic Centimeters.

All Animals Previously Fasted for Twenty-Four Hours.

No. of animal.	Weight in gm.	Urine in c.c.	Dextrose in urine in gm.	Hours,
A 142 ¹¹	1,160	105	0.00	24
A 143 ¹¹	1,430	118	>0.96	24
A 144 ¹¹	1,390	90	0.00	24
A 145 ¹¹	1,240	89	0.00	24
A 146 ¹¹	1,980	117	0.37	24
A 147 ¹¹	2,100	146	0.00	24
A 148 ^{11, 12}	1,860	59	0.60	24
A 149 ¹¹	1,890	51	0.34	24
A 150 ¹²	1,900	141	0.00	24
A 151 ¹²	1,690	151	1.12	24
A 152 ¹¹	1,620	106	1.05	24
A 153 ¹⁴	2,260	134	2.65	24
A 154 ¹⁴	2,010	80	2.09	24
A 155 ¹²	1,780	145	1.05	24
A 156 ¹¹	1,310	73	0.00	24
A 157 ¹²	1,620	69	0.35	24
A 158 ¹²	1,780	104	1.06	24
A 159 ¹¹	1,380	96	0.47	24
A 160 ¹¹	1,570	88	0.00	24
A 161 ¹²	1,680	45	0.28	24

Average of all, 100 c.c. urine, 0.62 gm. dextrose.

Average of 13 glycosurias, 98 c.c. urine, 0.95 gm. dextrose.

similar doses injected into one place. There is a clear difference in the results of the two tables, and the difference is definitely in favor of the single injections. While there were no failures in the subcutaneous injections in table II (and only one failure in table III with single subcutaneous injections of 0.7 of a cubic centimeter),

¹¹ Adrenalin, 0.7 c.c.

¹² Pregnant.

¹³ Adrenalin, 0.75 c.c.

¹⁴ Adrenalin, 0.8 c.c.

we find seven failures in table X; that is, seven rabbits out of twenty failed to show glycosuria after receiving subcutaneously a dose of 0.7 to 0.8 of a cubic centimeter injected into four different parts. Furthermore the average amount of dextrose in the urine after single injections (table II) was 1.13 grams, while the average amount of dextrose for the twenty rabbits in table X was only 0.62 of a gram; and even after deducting the seven rabbits with sugar-free urine, the average amount of dextrose for the remaining thirteen rabbits was only 0.95 of a gram. Finally, the average volume of urine for twenty-four hours, when the adrenalin was given subcutaneously in a single spot, amounted to 125 cubic centimeters, while the average amount of urine for the twenty animals which received their dose of adrenalin subcutaneously, distributed over four areas (table X), was only about 100 cubic centimeters. Evidently the distribution of a dose of adrenalin has the tendency to diminish the quantity of urine voided in twenty-four hours, to increase the number of cases in which no dextrose at all appears in the urine, and, when glycosuria is produced, to decrease the quantity of dextrose in the urine.

If we assume that the above mentioned observation of Meltzer holds good also for adrenalin, namely, that the distribution of an injected substance over several areas favors its absorption, we must come to the conclusion that the better the absorption of adrenalin the less effective are its glycosuric and diuretic actions.

DISCUSSION.

The starting point for our investigation was the earlier experiments of Meltzer and Auer with adrenalin. For three of the actions of that drug, the raising of the blood pressure, the dilatation of the pupil, and the muscular weakness, the intramuscular injections are more effective than the subcutaneous. Naturally it was anticipated that a similar effect would be found with respect to the glycosuric action of adrenalin. What was obtained was a diametrically opposite result, that is, glycosuria was brought on with greater certainty and, in most instances, with greater intensity by subcutaneous than by intramuscular injection.

The glycosuric action of adrenalin differs from some of its other known

actions in several respects. In the first place, there is the proportion between the injected quantity and the extent of the effect. For the hemodynamic principle it has been shown (11) that under proper conditions the same quantity of the drug can be relied upon to bring about the same degree of effect. The same seems to hold good to a considerable degree also for the dilatation of the pupils, as was recently shown by Joseph (12). Regarding the glycosuric effect Herter (13) stated that "within certain limits the quantity of the sugar excreted appears proportional to the dose of adrenalin." It seems that this is indeed true only "within certain limits." Herter himself says soon after, that "there is reason to believe that when a certain dose is followed by a large excretion of sugar, a larger dose would not have materially increased this output." In our experiments the average amount of dextrose in the urine decreased in general with the decrease of the dose injected, subcutaneously as well as intramuscularly. This is especially the case for the doses of 0.7, 0.5, 0.4, and 0.3 of a cubic centimeter for an animal. At the same time the individual amounts of the urinary dextrose varied considerably for each dose, so that in some instances the dose of only 0.3 has given rise to the presence of a quantity of sugar in the urine which was larger than that of other cases after an injection of 0.7 of a cubic centimeter. Judging by the figures in various protocols, similar conditions apparently prevailed in the experience of other investigators.

We may state here in passing that some of the discrepancies can perhaps be accounted for by the circumstances that the subcutaneous injections of some investigators may have been actually intramuscular, as it may readily happen that inadvertently the point of the needle instead of remaining in the subcutaneous tissue penetrated the muscular fascia and was therefore actually within a muscle. On the other hand, it may also happen that a needle passed through a muscle bundle and the injection was actually subcutaneous (that is, the injected fluid passed into the loose connective tissue), instead of being intramuscular. It was for the purpose of eliminating such errors that in our experiments the solid mass of the lumbar muscles was selected for the intramuscular, and the lower quadrants of the abdominal wall with its loose skin for the subcutaneous injection. However, occasionally such an error may have happened to us, and may perhaps account for one or the other case with a high intramuscular and a low subcutaneous glycosuria.

At any rate, it is evident that the exactness of the relation of adrenalin to glycosuria falls far behind that of the relation of adrenalin to its effects upon blood pressure.

Another striking difference is to be found in the length of the interval between the administration of the adrenalin and the appearance of the effect. The comparatively slow onset of the glycosuria can in no way be compared with the promptness of the rise of blood pressure following an injection of adrenalin or the promptness of the appearance of the dilatation of the pupil after the removal of the superior cervical ganglion.

A further noteworthy difference is the disappearance of the glycosuric effect after repeated injections of adrenalin and the persistence of the effect upon the blood pressure and upon the dilatation of the pupil. This disappearance after repeated injections has been observed and studied by Pollak (17) and others. The persistence of the blood pressure effect is a well known fact noted by various

writers. The persistence of the pupillary reaction to adrenalin has been established by numerous observations in this laboratory. Ganglionectomized rabbits received injections of adrenalin frequently for weeks and months, sometimes every day and sometimes even several times on the same day, without the pupil ever failing to react promptly, and practically with the original intensity.

Evidently the production of glycosuria by adrenalin is a more complicated process than is the rise of blood pressure or the dilatation of the pupil and it is more difficult to control the numerous factors involved.

In view of the foregoing, it should occasion no surprise to find a difference between the glycosuric effect of adrenalin and some of its other effects. We should have found occasion for surprise if we had been compelled to interpret the difference to mean that for the production of glycosuria adrenalin is more readily absorbed from the subcutaneous than from the intramuscular tissue, while with regard to its other effects adrenalin is better absorbed from the muscles. Such an interpretation is, indeed, not indispensable. As we have seen above, our results permit us to assume that even for the production of glycosuria, adrenalin enters the circulation with greater readiness from the muscle than from the subcutaneous tissue. For the understanding of the results we need only to assume further that the production of glycosuria is rather favored by a slow entrance of the injected adrenalin into the circulation. This assumption finds support in the fact that the distribution of the injected dose of adrenalin over several areas, a procedure which favors a more rapid entrance of the chemical into the circulation, tangibly impairs the production of glycosuria. With this as a basis there is no difference between the experience of Meltzer and Auer with regard to the other effects of adrenalin and the present results with reference to its glycosuric action; in both cases adrenalin is better absorbed from the muscular than from the subcutaneous tissues.

The difference which our experiments show to exist between the glycosuric effect and the other effects of adrenalin consists, then, if our view is correct, in the fact that, for instance, the hemodynamic and the pupillodilating actions of adrenalin are the more effective the more rapidly the adrenalin gets into the circulation, while the glycosuric action, on the contrary, manifestly becomes greater with the retardation of the absorption of the drug into the blood.

Is this view probable, and is there any other evidence in favor of it? In the experiments of Meltzer and Auer with the hemodynamic action of adrenalin, it was found that an intramuscular injection of adrenalin acts nearly as well as an intravenous administration. Is a subcutaneous injection of adrenalin more favorable to the production of glycosuria than an intravenous one? The above mentioned and frequently quoted investigations of Ritzmann (14) seem to speak against such a conclusion. Adopting the method of Kretschmer (15) which the latter used for the study of the blood pressure-raising effect of adrenalin, Ritzmann infused intravenously very dilute solutions of adrenalin and found that not only does this method produce glycosuria, but the amount of dextrose in the urine produced by it exceeds considerably the amount of dextrose in the urine produced by a subcutaneous injection of a much larger dose of adrenalin. Underhill (16) who repeated Ritzmann's experiments found that the intravenous infusion of adrenalin in unanesthetized rabbits causes no glycosuria at all, and that the glycosuria observed by Ritzmann was produced by the simultaneous use of urethane as an anesthetic. Underhill further noted that a subcutaneous injection of adrenalin is more likely to cause glycosuria than an intravenous dose of the same size. Pollak (17) stated that the experience of the pharmacological laboratory of the University of Vienna is that a single intravenous injection of adrenalin, in doses ranging from 0.1 to 1 milligram, does not produce glycosuria, while, as we have seen, some of these doses readily produce glycosuria by subcutaneous injection.

Hence, our conclusion that the favorable effect which we obtained by subcutaneous injection of adrenalin was due to the slow absorption, and that any means which promotes absorption may impair the production of glycosuria finds, therefore, ample support in the statements of Underhill and of Pollak. Moreover it is not contradicted by the findings of Ritzmann which were influenced, as is shown by Underhill, by the use of urethane.

The many failures in our experiments to produce a glycosuria after the intramuscular injections and the still larger number of failures from the subcutaneous injections distributed over several

areas find their ready interpretation in the view that in both instances the absorption into the circulation was so rapid as to imitate an intravenous injection.

Pollak found that intravenous injections, which fail to cause glycosuria, nevertheless cause hyperglycemia, the degree of the latter being insufficient to produce glycosuria in the absence of increased diuresis. He observed further that the hyperglycemia produced by the subcutaneous injection of a certain dose of adrenalin exceeds that caused by a similar dose administered intravenously. Pollak calls attention to the diuretic action of adrenalin and believes that the following rules regulate the relation of glycosuria to hyperglycemia and diuresis. When a hyperglycemia in rabbits is more than 0.15 per cent., but not above 0.25 per cent., and is not accompanied by an increased diuresis there will be no glycosuria. If this moderate hyperglycemia is accompanied by an increased diuresis or if the hyperglycemia exceeds 0.25 per cent., without diuresis, glycosuria results. Our experiments show that subcutaneous injection, which favors glycosuria, also generally favors an increase in the diuresis. At the same time it is quite evident that both processes are independent of each other to a considerable extent.

To the above we wish to add the following remark. While we are sure that a theory which attempts to explain completely the reasons why subcutaneous injections, which have to act through the circulation, are superior to direct intravenous injections, would be premature at present, since our knowledge of the facts in question is still too scant, we nevertheless believe that an analysis of the phenomena should also take into consideration the action of adrenalin upon the process of elimination. Meltzer and Auer (18) previously pointed out that adrenalin restricts absorption as well as elimination, and they indicated that it may interfere with the eliminating power of the kidney. Drummond (19) has shown that adrenalin causes a (reversible) cloudy swelling of the kidney epithelium. Ritzmann (20) stated that when the intravenous infusion of the adrenalin solution took place too rapidly, no sugar appeared in the urine and there was, indeed, little or no urine. When the animal recovered from the effects of such an infusion it passed

some urine which often contained blood. From our personal experience we are confident that larger doses of adrenalin interfere with the elimination of sugar as well as of urine. For this reason we did not employ doses exceeding one milligram of adrenalin. The most satisfactory results relating to the presence of dextrose and to the volume of urine were obtained by us from doses of 0.7 of a cubic centimeter of the adrenalin solution. We believe, therefore, that beyond a certain point the increase of the dose of adrenalin lessens the elimination of the dextrose and restricts the diuresis. The size of the restricting dose is probably not the same for both functions. The influences of adrenalin upon diuresis and upon glycosuria (both the stimulating and restricting influences) are, we think, two essentially independent processes.

Without offering any theory of our own, we wish merely to state our belief that any theory which attempts to explain the phenomena under discussion should take into consideration the influence of adrenalin upon elimination of dextrose, as well as of urine.

SUMMARY.

Subcutaneous injections of adrenalin are, in contrast with its behavior in the production of the other effects of that drug, more favorable to the production of glycosuria in rabbits than intramuscular injections; the failures are fewer and the quantities of dextrose in the urine are generally larger. In general, as regards the stimulation of diuresis by adrenalin, a subcutaneous injection exerts generally a greater effect than an intramuscular one.

Subcutaneous injections of a certain dose of adrenalin distributed over several areas are far less effective than the administration of that dose in a single injection; they fail frequently to produce any glycosuria, the quantity of dextrose in the urine, when present, being less, and the quantity of urine being generally diminished.

Apparently the more slowly the injected adrenalin reaches the blood, the greater is its effect in producing glycosuria and generally, also, the greater its diuretic action.

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A CONTRIBUTION TO THE RELATION BETWEEN PROTEID CLEAVAGE PRODUCTS AND ANAPHYLAXIS.*

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The leading hypothesis among the attempts to explain proteid sensitization, or anaphylaxis, is that of Vaughan.¹ This view was advanced by Vaughan and Wheeler as early as 1907 and has, with more or less marked modifications, received wide acceptance.²

His conception may be summarized as follows: The parenteral introduction of a foreign proteid (sensitization) develops in the animal a proteolytic ferment specific for the proteid introduced. This specific ferment remains in the cells of the sensitized animal as a zymogen and is activated by the second injection of the same proteid. The zymogen itself is conceived not as a new substance in the cell but as a more or less permanent alteration of the atomic structure of the cell proteid molecule. A sensitized animal, therefore, is one whose cells are rich in a specific proteolytic zymogen. As Vaughan conceives all proteids to be composed of a toxic and a non-toxic fraction, and as the injection of a foreign proteid into a sensitized animal brings about activation of the specific zymogen, the active ferment thus formed frees the toxic portion of the proteid molecule and these poisonous substances cause the picture of "anaphylactic intoxication." The first injection of the proteid causes no symptoms because the cleavage or digestion of the foreign proteid by the body cells takes place so slowly that at no one time is there a sufficient amount of the toxic proteid group to cause symptoms. Vaughan also holds the view that "each foreign proteid has its predilection tissue in which it is largely deposited, whose cells it especially sensitizes, and where it is disrupted."³

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¹ Vaughan, V. C., *Am. Jour. Med. Sc.*, 1913, cxlv, 161.

² We shall not enter into a historical review of this matter, nor do we wish to deny that other investigators may have reached similar views independently of Vaughan. It seems to us, however, that Vaughan has the credit of giving the first sharp, clean cut conception, based on experimental evidence, of the rôle which cleavage products may play in proteid sensitization.

³ Vaughan, V. C., *loc. cit.*, p. 176.

These views have stimulated investigators to the search for proteolytic ferments and proteid cleavage products both in the blood of fully sensitized animals and in animals during the anaphylactic reaction. We shall not enter into a criticism of the many interesting and contradictory results which have been obtained; for these the reader is referred to the works of Abderhalden,⁴ Pfeiffer and Mita,⁵ Zunz,⁶ and Doerr.⁷ We shall merely state that no conclusive evidence has yet been obtained that cleavage products of the reinjected proteid produce the symptoms of the anaphylactic reaction.⁸

In the search for proteolytic ferments and cleavage products the investigators limited themselves to one field, the blood. As far as we are aware no one has sought for them in the specific tissues where active anaphylaxis manifests itself by characteristic functional and anatomical changes, a location, therefore, where these substances theoretically must be present. There are two organs especially available for such an investigation, the anaphylactic lung of the guinea pig⁹ and the anaphylactic heart of highly sensitized rabbits.¹⁰ In both instances these organs are respectively the immediate primary cause of death, and, therefore, on the basis of the theory briefly outlined above, enough cleavage products of one kind or another must be present in the substance of these organs themselves to produce the characteristic changes which bring about acute death. Immediate examination of these organs after death ought, therefore, to reveal them, provided that cleavage products are present, and that their amount is not beyond the range of the method employed.

These considerations and the fact that an exceedingly delicate

⁴ Abderhalden, E., *Die Schutzfermente des tierischen Organismus*, Berlin, 1912; *Ztschr. f. physiol. Chem.*, 1912, lxxxii, 109.

⁵ Pfeiffer, H., and Mita, S., *Ztschr. f. Immunitätsforsch., Orig.*, 1910, vi, 18.

⁶ Zunz, E., *Ztschr. f. Immunitätsforsch., Orig.*, 1913, xvii, 241.

⁷ Doerr, R., in Kolle and Wassermann, *Handbuch der pathogenen Mikroorganismen*, 2d edition, Jena, 1913, ii, 1030.

⁸ For a full discussion see Zunz, E., *loc. cit.*, pp. 288-298.

⁹ Auer, J., and Lewis, P. A., *Jour. Am. Med. Assn.*, 1909, liii, 458; *Jour. Exper. Med.*, 1910, xii, 151.

¹⁰ Auer, J., *Zentralbl. f. Physiol.*, 1910-11, xxiv, 957; *Jour. Exper. Med.*, 1911, xiv, 476.

method for the detection of proteid cleavage products was available¹¹ led us to investigate the problem. For convenience we chose as the material for examination the characteristic lungs of guinea pigs that died acutely from serum anaphylaxis.

EXPERIMENTS.

Thirty-seven guinea pigs were used in all. Of these sixteen were normal controls and twenty-one had been sensitized by the subcutaneous injection of 1 c.c. of horse serum. After intervals of 23, 35, 43, 54, and 57 days the sensitized animals were stretched out on an electric pad to prevent loss of heat, were lightly etherized and a cannula was tied into an external jugular vein. After full recovery from the ether, either 0.5 or 0.9 c.c. of warm horse serum¹² was injected into the jugular cannula and the cannula was then washed clear by a following injection of 1.5 c.c. of Ringer or salt solution. The large majority of the sensitized animals showed typical symptoms and died three to six minutes after the intravenous injection. Three did not die acutely, but were killed by severing the neck. The animals were autopsied immediately after cessation of respiration, and the lungs excised. Their appearance was typical in the cases that succumbed acutely: the lungs remained in full inspiratory position on excision, the surfaces and borders were smooth, the color was pale bluish red; no foam was in the trachea; and, as a rule, no hemorrhages were visible. The lungs were then freed from the trachea and the mass of tissue at the hilus, the various lobes cut through a number of times, swiftly washed off with Ringer solution, dried quickly with filter paper, and then examined at once or cooled at 0° C. until they could be used, which was never later than after two hours. The lungs were not allowed to remain for any length of time at room temperature.

The lungs of the three sensitized animals which were killed ten minutes after reinjection of the horse serum were also found to remain distended after excision from the chest, but their distension was not as marked, and their appearance was less firm than that of the other lungs.

The controls were treated in exactly the same manner: they were stretched out on the electric pad, lightly etherized, and a jugular cannula was introduced; after a few minutes 0.9 c.c. of warm horse serum was injected and the cannula washed clear by 1.5 c.c. of Ringer or salt solution. The horse serum caused no noticeable effect in the controls. About five minutes after the injection, the animals were killed by injecting 4 c.c. of air into the jugular vein. Two controls were killed by severing the neck. On immediate autopsy the lungs of all controls collapsed practically fully on excision and formed a small grayish mass which differed strikingly from the anaphylactic lung. Their further preparation was the same as that of the anaphylactic lungs.

¹¹ Van Slyke, D. D., *Jour. Biol. Chem.*, 1912, xii, 275.

¹² The horse serum employed was about nine months old.

ANALYTICAL METHODS.

Amino Nitrogen.—The lungs were weighed to within 0.01 gm., then coagulated with hot water, minced fine, and further extracted several times with hot water. The extracts were concentrated *in vacuo*, and a small amount of protein, not coagulable by heat, was precipitated with methyl alcohol. The filtrate was then concentrated *in vacuo* to remove the alcohol. The residue was diluted to 10 c.c. in a measuring flask, and portions of 2 c.c. were used for determination of amino nitrogen by Van Slyke's method,¹³ a small apparatus being used, which permitted measurement of the nitrogen gas to within 0.01 c.c. (table I).

Total Non-Coagulable Amino Nitrogen (Free NH_2 of Amino Acids, Peptones, Albumoses, etc.).—The hot water extracts were concentrated *in vacuo* to 10 c.c. and used directly for determination of amino nitrogen, without alcohol treatment. The results give the total nitrogen of the α -amino acids, and the end NH_2 of the higher products (table II).

Non-Coagulable Amino Nitrogen after Hydrolysis.—5 c.c. of the above solution were mixed with 5 c.c. of hydrochloric acid (specific gravity 1.18) and heated for twenty-four hours at 100° C. This treatment ensures complete hydrolysis.¹⁴ The acid was removed by concentration *in vacuo* as completely as possible. The residue was taken up in water, to which a slight excess of suspended calcium hydrate was added. The solution was then again concentrated *in vacuo* to remove the ammonia which is always formed on hydrolysis of animal proteins. The residue was brought to 10 c.c., and portions of 2 c.c. were used for amino nitrogen determination. The latter gives about 75 per cent. of the total nitrogen of the non-coagulable proteins and intermediate products (albumoses, peptones, etc.¹⁵ (table II)).

The experimental basis of the methods will be discussed in connection with another article. The results are accurate to within 1 or 2 mg. of amino nitrogen per 100 gm. of fresh tissue. Samples of the same organ from different animals of the same species vary over a much wider range, however, as is shown by the results tabulated. Most of the analyses were made in duplicate.

RESULTS.

The following tables give the results of this investigation. It must again be noted that the analytical data of table I cannot be compared directly with those of table II, because the lung tissues were treated differently before analysis. The data of table I give the amino nitrogen from compounds not precipitated by alcohol; these are chiefly the simple amino acids. The analytical data in table II represent the free amino nitrogen of the compounds not

¹³ Van Slyke, D. D., *loc. cit.*

¹⁴ Van Slyke, D. D., *loc. cit.*, p. 295.

¹⁵ The other 25 per cent. belongs to the ammonia and the amino acids which contain part (tryptophane, arginine, histidine) or all of their nitrogen (proline, oxyproline) in other than amino groups.

TABLE I.

Anaphylaxis.

All guinea pigs were sensitized by the subcutaneous injection of 1 c.c. of horse serum. The reinjection dose was always given into the external jugular vein.

No. of animal.	Sex	Weight.	Length of sensitization.	Reinjection dose.	Death after	Amino nitrogen per 100 gm. of lung tissue.	Remarks.
I 7	F.	360 gm.	23 dys.	0.5 c.c.	6 min.	31 mg.	Not pregnant.
I 8	M.	380 gm.	23 dys.	0.5 c.c.	10 min.	25 mg.	Killed by cutting cervical cord.
I 9	M.	365 gm.	23 dys.	0.9 c.c.	3 min.	27 mg.	
I 10	M.	335 gm.	23 dys.	0.9 c.c.	3 min.	25 mg.	
I 11	M.	405 gm.	23 dys.	0.9 c.c.	10 min.	25 mg.	Killed by cutting cervical cord.
I 12	F.	455 gm.	23 dys.	0.9 c.c.	3 min.	20 mg.	Very early pregnancy.
H 189	M.	520 gm.	43 dys.	0.5 c.c.	14 min.	20 mg.	
H 190	—	470 gm.	43 dys.	0.9 c.c.	3 min.	28 mg.	
I 1	M.	745 gm.	57 dys.	0.5 c.c.	5 min.	Lost	
I 2	F.	535 gm.	57 dys.	0.5 c.c.	5 min.	23 mg.	Not pregnant.
I 3	M.	465 gm.	57 dys.	0.5 c.c.	5 min.	20 mg.	

Total average: 24.4 mg. of amino nitrogen per 100 gm. of lung tissue.

Normal Controls for Table I.

The animals were killed by injecting air into the jugular vein.

No. of animal.	Sex	Weight.	Dose of serum.	Method of death.	Time of death after serum.	Amino nitrogen per 100 gm. of lung tissue.	Remarks.
a H 192	M.	385 gm.	0.9 c.c.	10 c.c. of air	6 min.	32 mg.	The air was always injected 5 min. after the horse serum.
b H 192	M.	315 gm.	0.9 c.c.	10 c.c. of air	6 min.	25 mg.	
c H 192	F.	375 gm.	0.9 c.c.	10 c.c. of air	6 min.	23 mg.	Not pregnant.
I 4	M.	265 gm.	0.5 c.c.	4 c.c. of air	6 min.	30 mg.	
I 5	F.	290 gm.	0.5 c.c.	2 c.c. of air	6 min.	28 mg.	Very early pregnancy.
I 6	M.	245 gm.	0.5 c.c.	4 c.c. of air	6 min.	36 mg.	

Total average of controls: 29 mg. of amino nitrogen per 100 gm. of lung tissue.

coagulated by heat, and give not only the amino nitrogen of the amino acids but also that of peptones, albumoses, etc. The figures represent all the nitrogen of the simple α -amino acids, 6 to 10 per cent. nitrogen of the albumose, and up to 50 per cent. of that of the simpler intermediate products, such as peptones and peptids. The analytical data in the last column of table II give the amino nitrogen obtained after converting all these intermediate products into amino acids by complete hydrolysis with hydrochloric acid.

A study of the tables shows that the lungs of guinea pigs which died of typical acute anaphylaxis did not yield more amino nitrogen in the three procedures employed than the lungs of normal control animals. Thus in table I ten anaphylactic animals yielded on the average 24.4 milligrams of amino acids per 100 grams of lung tissue, while six control animals gave 29 milligrams for the same amount of tissue; amino acids were therefore surely not more abundant in the anaphylactic than in control lungs. In table II where the free amino nitrogen not only of the amino acids but also of the peptones, albumoses, etc., was determined, we find the average after acute anaphylactic death (ten animals) to be 61.8 milligrams per 100 grams of lung tissue, while controls (ten animals) show 58.5 milligrams for the same amount of tissue; the small difference between the two averages (3 milligrams) is without significance, as it falls within the range of normal variation. When the non-coagulable amino nitrogen after hydrolysis with hydrochloric acid was determined in five anaphylactic and five control lungs the results again showed no significant differences: the average yield of the anaphylactic lungs per 100 grams of tissue was 172.6 milligrams, while the average for the controls was 171.2 milligrams.

Especial attention may be called to the significance of the data obtained after hydrolysis. If there were a difference in the content of albumoses, peptones, etc., in the lungs of the anaphylactic animals as compared with those of the controls, this discrepancy should be increased because the analysis after complete hydrolysis determines not only the free amino acid nitrogen of the albumoses, peptones, etc., but also the amino acid nitrogen of the amino acids formed from the albumoses, peptones, etc., by the process of hydrolysis; much larger quantities of amino acid nitrogen are thus obtained for

TABLE II.
Anaphylaxis.

The animals were prepared as for table I, except that the lungs were treated for analysis in a different way (see text, page 213).

No. of animal.	Sex.	Weight.	Length of sensitization.	Reinjection dose.	Death after	Amino nitrogen not coagulable by heat.		Remarks.
						Without hydrolysis.	After hydrolysis.	
I 19	F.	485 gm.	35 dys.	0.9 c.c.	3 min.	72 mg.		Early pregnancy. Killed by cutting cervical cord.
I 20	M.	395 gm.	35 dys.	0.9 c.c.	9 min.	75 mg.		
I 21	M.	405 gm.	35 dys.	0.9 c.c.	4 min.	63 mg.		
I 22	M.	440 gm.	35 dys.	0.9 c.c.	3 min.	62 mg.		
I 23	M.	440 gm.	35 dys.	0.9 c.c.	6 min.	67 mg.		
I 35	F.	510 gm.	54 dys.	0.9 c.c.	3½ min.	58 mg.	168 mg.	Early pregnancy. Early pregnancy. Early pregnancy. Late pregnancy. Early pregnancy.
I 36	F.	317 gm.	54 dys.	0.9 c.c.	4 min.	52 mg.	150 mg.	
I 37	F.	520 gm.	54 dys.	0.9 c.c.	4 min.	52 mg.	170 mg.	
I 38	F.	490 gm.	54 dys.	0.9 c.c.	5 min.	56 mg.	173 mg.	
I 39	F.	370 gm.	54 dys.	0.9 c.c.	3½ min.	61 mg.	202 mg.	
Total average						61.8 mg. of free amino nitrogen per 100 gm. of tissue	172.6 mg. amino nitrogen after hydrolysis per 100 gm. of lung tissue	

Normal Controls for Table II.

No. of animal.	Sex.	Weight.	Dose of serum.	Method of death.	Time of death after serum.	Amino nitrogen not coagulable by heat.		Remarks.
						Without hydrolysis.	After hydrolysis.	
I 24	M.	345 gm.	0.9 c.c.	4 c.c. of air in jugular vein	8 min.	58 mg.		Not pregnant. Not pregnant. Not pregnant.
I 25	M.	280 gm.	0.9 c.c.	4 c.c. of air in jugular vein	9 min.	61 mg.		
I 26	M.	254 gm.	0.9 c.c.	Cord cut	8 min.	53 mg.		
I 27	M.	284 gm.	0.9 c.c.	Cord cut	7 min.	52 mg.		
I 28	M.	304 gm.	0.9 c.c.	4 c.c. of air	9 min.	60 mg.		
I 40	M.	385 gm.	0.9 c.c.	4 c.c. of air	8 min.	62 mg.	185 mg.	
I 41	M.	390 gm.	0.9 c.c.	4 c.c. of air	6 min.	61 mg.	163 mg.	
I 42	F.	265 gm.	0.9 c.c.	4 c.c. of air	7½ min.	59 mg.	178 mg.	
I 43	F.	320 gm.	0.9 c.c.	4 c.c. of air	6 min.	60 mg.	155 mg.	
I 44	F.	315 gm.	0.9 c.c.	4 c.c. of air	7 min.	59 mg.	175 mg.	
Total average						58.5 mg.	171.2 mg.	

determination, and any difference in the content of these substances in the two types of lung should therefore be more easily detectable. As a matter of fact the differences between the averages of the two kinds of lungs were negligible.

The conclusion therefore is fully warranted that the amino acid, peptone, and albumose content of lungs which show undoubted anaphylactic changes is apparently identical with that of normal controls. Thus our results do not support the proteid cleavage theory of anaphylaxis, yet they do not exclude it, because it is conceivable that specifically toxic cleavage products may be formed in such minute amount that they do not appreciably influence the total quantity of cleavage products normally present.

It may be worthy of notice that fluctuations in the amino nitrogen figures amounting to one third of the amount present may occur in animals which were kept under approximately the same conditions and that neither sex nor pregnancy exerted any definite effect on the content of amino nitrogen in the lung.

SUMMARY.

Examination of the anaphylactic lung by means of Van Slyke's amino nitrogen method gave no evidence of an increased amount of proteid cleavage products of any kind in this organ. This investigation gives no support to the hypothesis that the true anaphylactic lung of the guinea pig is caused by proteid split products.

THE INFLUENCE OF TEMPERATURE ON THE ACTION OF STRYCHNIN IN FROGS.*

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INTRODUCTION.

There is a widespread assumption that in cold blooded animals warming increases the action of all drugs. It is repeatedly pointed out that all chemical, and therefore, presumably, all vital, processes are increased in intensity with rise of temperature, and that the reaction to all drugs is therefore also increased.

Thus Guinard (1) says: "The influence of warmth on therapeutic action is well known. Cl. Bernard stated it long since, pointing out that the sensitiveness of the frog to poisons increases in proportion as the temperature rises. It is a fact which is perfectly logical, because chemical action is the basis of all response to drugs and chemical reactions in general occur more rapidly the higher the temperature. When one administers strychnin to frogs which are each exposed to different temperatures, one notices that rise in temperature accelerates and increases the convulsions." Richet (2) uses similar language. He says: "The influence of temperature on intoxication is particularly marked in cold blooded animals, whose temperature is variable. . . . At a low temperature chemical actions are less intense, and as toxic actions are merely chemical actions, it follows that at a low temperature poisons are less active than at high temperatures. . . . The action of temperature on toxic action is evidently a very general law . . . which may be formulated thus: Toxic actions being chemical actions vary with the temperature of the animal. They are the more active the higher is the temperature of the animal."

In the text-books on pharmacology and physiology there is usually no general statement in regard to the influence of temperature, but in medical journals we meet with the same general view applied to some particular drug. Thus Luchsinger (3), speaking of picrotoxin, refers to Kunde's conclusions, which will be discussed below, and says that summer frogs show much stronger convulsions than winter frogs, and "it seems plausible (*liegt nahe*) to seek in difference of temperature the cause of the great difference in behavior of summer and winter frogs." Schlick (4) says: "Frogs react less to strychnin in the winter, when my experiments were done." The only article, however, which deals directly with the influence of temperature on the action of strychnin in frogs is that of

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Kunde (5), referred to by Luchsinger, which we shall quote at more length. Kunde's experiments were suggested by the fact that frogs react to weaker electric currents when warm, and therefore the cord is less sensitive in the cold and should be less affected by equal amounts of strychnin. He put three frogs in a 1:5,000 solution of strychnin nitrate at 16° C. for ten minutes and then transferred them to dishes containing water at respectively 31° C., 16° C., and 1° C. In ten minutes the frog at 31° C. had tetanus. After fifteen minutes the frog at 16° C. had no tetanus; it was transferred to 31° C. and had tetanus in fifteen minutes. The frog at 1° C. had no tetanus in thirty-five minutes; it was transferred to 31° C. and had tetanus in twelve minutes. The next morning all three appeared normal, but on being put between lumps of ice they developed tetanus, which disappeared when they were warmed, and returned when they were again cooled. On the basis of these experiments Kunde formulated the following law: "With a small dose of strychnin application of warmth stops the tetanus, withdrawal of warmth causes it. With a larger dose warmth brings on tetanus, withdrawal of heat prevents its occurrence." That he considers the effect of temperature on large doses of most importance is plain from his introductory remark. "If a frog is cooled, an electric current which before produced tetanus leaves the animal unaffected. . . . As it has been shown that the cord is also a reagent for strychnin, its effect on the spinal cord must change, if with a constant amount of strychnin present, the temperature of the nerve centers is changed. This hypothesis has, I believe, been confirmed."

From a series of experiments in which we have studied the influence of temperature upon the convulsant action of strychnin, it became evident that the relation of temperature to the tetanic effect of strychnin is erroneously presented when merely expressed in general statements. Neither does an observation of the animal limited to a few hours tell the entire story. In the following we shall report briefly the results which we have obtained in a more detailed study of the influence of temperature upon strychnin tetanus in frogs.

EXPERIMENTAL DATA.

The experiments were performed during the months of January and February on *Rana pipiens*, weighing between fifteen and thirty grams. After injecting in a femoral or the dorsal lymph sac a certain dose of strychnin, the frogs were put in appropriate cages where they were kept at constant, definite temperatures, ranging from 5° C. to 30° C., and observed until recovery took place or several days had passed.

Minimum Convulsant Dose.—In these experiments larger doses, that is, doses which are larger than a reliable minimum, were not

used. Only the effect of minimum doses was studied. But, as will be seen presently, the minimum convulsant dose varies with the temperature at which the animal is kept. A dose which proves to be a reliable minimum at a certain temperature may prove unreliable at another. Only with doses of 0.0006 of a milligram per gram (0.015 to 0.02 of a milligram per frog of twenty-five to thirty grams) did all the frogs show, sooner or later, tetanus at all temperatures. With 0.0005 of a milligram per gram all frogs had tetanus at 5° C., 27° C., and 30° C.; four out of six had tetanus at 13° C., two out of six at 18° C., two out of six at 24° C., and none (of six) at 21° C. With 0.0003 of a milligram per gram all frogs had tetanus at 5° C., while it was inconstant at temperatures above 24° C. and was never seen between 13° and 21° C. With 0.0002 of a milligram per gram three frogs out of five showed tetanus at 5° C., and none at any higher temperature. With smaller doses tetanus was not obtained. That the tetanus occurring at 30° C. is not a heat tetanus is shown by the fact that controls kept at this temperature showed no deviation from normal.

From the above we learn that with a dose of strychnin of 0.0005 of a milligram per gram of frog, only at the low temperature of 5° C. and at the high temperatures of 27° and 30° C. was there a tetanic response in every animal tested, while at various intermediary temperatures the dose failed to be effective in some cases, and at 21° C. failed in every instance. In other words, *a certain dose may be effective at high and low temperatures and be unreliable or fail completely at an intermediary temperature.* This demonstrates the incorrectness of a general statement that strychnin tetanus is favorably or unfavorably influenced by increase of temperature.

We shall reproduce here several abbreviated protocols of experiments with doses of 0.0005 and 0.0003 of a milligram per gram of frog, which may illustrate various points to be brought out in this paper.

In the first experiments a dose of strychnin sulphate equal to 0.0005 of a milligram per gram of frog was injected into the femoral lymph sac.

Frog 102.—Female; weight, 31 gm. Temperature 5° C. January 16, 1913, 9.16 A.M. Injected 0.155 c.c. of strychnin sulphate, 0.01 per cent. 11.30 A.M. Slightly hyperesthetic. 1.00 P.M. On touch the legs fly out in extension and are immediately drawn in. 5.00 P.M. The same. January 17, 9.00 A.M. Animal found in violent continuous tetanus which lasted two days and then gradually passed off. *At 5° C. violent tetanus developed more than 8 hours after injection and lasted for two days.*

Frog 105.—Male; weight, 22 gm. Temperature 5° C. January 16, 1913, 9.54 A.M. Injected 0.11 c.c. of strychnin sulphate, 0.01 per cent. 11.30 A.M. Distinct hyperesthesia. 1.00 P.M. Momentary extension on touch. 5.00 P.M. The same. January 17, 9.00 A.M. In violent continuous tetanus. January 18. The same. January 19. The same. January 20, 9.00 A.M. Still in strong tetanus. 5.00 P.M. Tetanus somewhat less violent. January 21. Markedly spastic but no tetanus. *At 5° C. strong tetanus appeared the day after the injection and continued for about four days.*

Frog 107.—Male; weight, 22 gm. Temperature 13° C. January 16, 1913, 9.22 A.M. Injected 0.11 c.c. of strychnin sulphate, 0.01 per cent. 10.50 A.M. On touch the legs are thrown out stiffly and at once drawn in. 1.00 P.M. Tetanus of brief duration; legs then drawn in. 2.25 P.M. Attacks of longer duration. 5.00 P.M. Strong prolonged attacks of extensor tetanus spontaneously; legs drawn in after attacks. January 17, 9.00 A.M. Almost normal. *At 13° C. tetanus appeared on the day of the injection and was gone the next day.*

Frog 108.—Male; weight, 22 gm. Temperature 13° C. January 16, 1913, 9.56 A.M. Injected 0.11 c.c. of strychnin sulphate, 0.01 per cent. 10.25 A.M. Slightly spastic. 11.05 A.M. Momentary strong extension. 2.00 P.M. The same. 4.00 P.M. The same. 5.00 P.M. The same. January 17, 9.00 A.M. Almost normal. *At 13° C. hardly any tetanus; normal the next day.*

Frog 109.—Male; weight, 25 gm. Temperature 21° C. January 15, 1913, 1.18 P.M. Injected 0.105 c.c. of strychnin sulphate, 0.01 per cent. 1.45 P.M. Slight hyperesthesia. 2.10 P.M. Markedly spastic. 3.00 P.M. The same. 4.00 P.M. The same. January 16, 9.00 A.M. Normal. *At 21° C. no definite tetanus at any time.*

Frog 100.—Male; weight, 20 gm. Temperature 24° C. January 15, 1913, 1.15 P.M. Injected 0.1 c.c. of strychnin sulphate, 0.01 per cent. 1.45 P.M. Slight hyperesthesia. 2.00 P.M. More spastic. 3.40 P.M. Markedly spastic. 5.00 P.M. Slightly spastic. January 16, 9.00 A.M. Normal. *At 24° C. no tetanus at any time.*

Frog 110.—Male; weight, 22 gm. Temperature 27° C. January 16, 1913, 9.36 A.M. Injected 0.11 c.c. of strychnin sulphate, 0.01 per cent. 9.50 A.M. Very lively and active. 10.05 A.M. The same. 10.25 A.M. Sudden strong extensor tetanus; legs not drawn in. 12.00 M. Still in strong tetanus. 1.00 P.M. Less violent. 2.25 P.M. Having attacks of extensor tetanus, but legs drawn in between attacks. 4.00 P.M. Extremely spastic. January 17, 9.00 A.M. Normal. *At 27° C. tetanus in 49 minutes; normal the next day.*

Frog 113.—Female; weight, 30 gm. Temperature 27° C. January 16, 1913, 10.50 A.M. Injected 0.15 c.c. of strychnin sulphate, 0.01 per cent. 11.15 A.M. Spastic. 11.30 A.M. Sudden attack of strong extensor tetanus; legs not drawn in. 4.30 P.M. Becoming less violent. January 17, 9.00 A.M. Almost normal. *At 27° C. tetanus after 40 minutes; normal the next day.*

Frog 128.—Female; weight, 26 gm. Temperature 30° C. January 20, 1913,

11.18 A.M. Injected 0.13 c.c. of strychnin sulphate, 0.01 per cent. 11.45 A.M. Slightly spastic. 11.53 A.M. Extensor tetanus; legs drawn in between attacks. 12.10 P.M. Strong extensor tetanus; legs not drawn in. 12.30 P.M. More violent. 1.00 P.M. Becoming less violent. 3.00 P.M. Recovering. Merely moderately spastic. *At 30° C. tetanus after 35 minutes, recovering the same day.*

In the following experiments strychnin was administered in doses of 0.0003 of a milligram per gram of frog.

Frog 31.—Female; weight, 48 gm. Temperature 5° C. January 7, 1913, 9.41 A.M. Injected 0.48 c.c. of strychnin sulphate, 0.003 per cent. 10.15 A.M. On touch the legs are thrown out stiffly and then drawn in. 4.30 P.M. Condition the same. January 8, 9.00 A.M. In strong continuous extensor tetanus. 2.00 P.M. Still in tetanus. *At 5° C. strong tetanus the next day.*

Frog 144.—Male; weight, 20 gm. Temperature 5° C. January 20, 1913, 5.11 P.M. Injected 0.2 c.c. of strychnin sulphate, 0.003 per cent. January 21, 8.00 A.M. In continuous strong extensor tetanus. 4.00 P.M. The same. 6.00 P.M. Legs drawn in at times. January 22, 8.00 A.M. On touch extensor tetanus lasting several seconds, then legs drawn in. 5.30 P.M. The same. January 23, 9.00 A.M. On touch momentary strong extension. 2.00 P.M. Markedly spastic. 5.00 P.M. Moderately spastic. January 24, 9.00 A.M. Normal. *At 5° C. onset of tetanus not later than 15 hours; lasted several days.*

Frog 74.—Female; weight, 26 gm. Temperature 13° C. January 10, 1913, 9.36 A.M. Injected 0.26 c.c. of strychnin sulphate, 0.003 per cent. 10.00 A.M. Slight hyperesthesia. 11.00 A.M. Slightly spastic. 3.00 P.M. More spastic. 5.00 P.M. The same. January 11, 9.00 A.M. Still spastic. 5.00 P.M. Normal. *At 13° C. no tetanus.*

Frog 82.—Female; weight, 25 gm. Temperature 25° C. January 13, 1913, 11.32 A.M. Injected 0.25 c.c. of strychnin sulphate, 0.003 per cent. 12.05 P.M. Slight hyperesthesia. 12.25 P.M. Markedly spastic. 4.30 P.M. Less spastic. January 14, 9.00 A.M. Normal. *At 25° C. no tetanus.*

Frog 130.—Male; weight, 21 gm. Temperature 30° C. January 20, 1913, 12.13 P.M. Injected 0.21 c.c. of strychnin sulphate, 0.003 per cent. 12.23 P.M. Slight hyperesthesia. 12.30 P.M. The same. 1.00 P.M. In strong continuous extensor tetanus. 1.30 P.M. The same. 3.00 P.M. Legs drawn in between attacks. 4.00 P.M. Markedly spastic; no more tetanus. 4.30 P.M. Slightly spastic. *At 30° C. tetanus after 47 minutes, with full recovery on same day.*

The Length of the Interval between the Injection of Strychnin and the Onset of Tetanus.—The foregoing protocols bring out in the first place the fact already alluded to; namely, that a low temperature as well as a high one may bring on tetanus which may fail to appear at an intermediary temperature. The protocols, however, show also that the length of the interval between the injection and the onset of a definite tetanus seems to be in inverse proportion to the degree of temperature. For instance in experiments 102 and

105, in which the animals were kept at 5° C., a definite tetanus appeared the next day or at least many hours after the injection, while in experiments 110, 113, and 128, in which the animals received the same dose of strychnin (0.0005 per gram of frog), but were kept at 27° C. or 30° C., the interval was less than one hour. Such a relation may be of especial interest, since most of the earlier observations seem to have been based largely upon the early occurrence of tetanic symptoms. This may be the reason why the tetanic effect at low temperatures has been frequently overlooked. In our experiments the animals were kept under observation for several days and the late appearance of tetanic manifestations was scrutinized. Table I shows that while it is true in a general way that high temperatures tend to shorten and low temperatures to lengthen the interval, the figures in the various experiments are too irregular to make them fit into a definite general rule.

Duration of the Tetanic State.—In our experiments notes were taken also regarding the duration of the tetanic state under different temperatures. Here again it may be said, in a general way, that at lower temperatures the duration of the tetanic state is longer than when the animal is kept at higher temperatures. The figures, however, are too variable to press them into a definite rule, as will be seen on examination of the figures in table I. We may note that at 13° C. with doses of 0.0005 and 0.0006 of a milligram per gram tetanus always came on within a few hours and passed off during the following night, while with 0.0003 of a milligram per gram no tetanus was obtained, so that there are no observations on the exact duration of tetanus at this temperature.

Character of the Tetanus.—There is a marked difference in the behavior of the tetanic frog at low temperatures and that at high. In the cold each attack is of long duration, and in the interval between them the animal seems to remain in a certain state of increased tonus. The forelegs are held drawn against the chest, and if the hind legs are flexed passively they extend at once. In a frog having tetanus at a high temperature the attacks are much briefer and in the interval between the attacks there is complete relaxation. When an animal is stimulated soon after an attack has passed off,

TABLE I.

Table Showing for Each Dose and Each Temperature the Number of Frogs Injected, the Number Showing Distinct Tetanus, the Interval between the Injection and the Onset of Tetanus, and the Duration of Tetanus.

Dose 0.0003 mg. per gm.			Dose 0.0005 mg. per gm.			Dose 0.0006 mg. per gm.		
Tempera- ture.	No. of ex- periments.	Tetanus in.	Tempera- ture.	No. of ex- periments.	Tetanus in.	Tempera- ture.	No. of ex- periments.	Tetanus in.
5° C.	11	11	5° C.	10	10	5° C.	10	10
No. of frog.	Before onset.	Duration.	No. of frog.	Before onset.	Duration.	No. of frog.	Before onset.	Duration.
28	144 min.	—	102	7-24 hrs.	—	148	31 min.	—
31	95 min.	—	103	7-24 hrs.	—	149	29 min.	—
32	93 min.	—	104	7-23 hrs.	—	150	34 min.	—
76	8-23 hrs.	—	105	7-23 hrs.	—	151	32 min.	—
77	8-23 hrs.	—	192	7-27 hrs.	—	164	160 min.	—
88	5-21 hrs.	—	193	25 hrs.	—	165	158 min.	—
89	5-21 hrs.	—	222	5 hrs.	80 hrs.	178	66 min.	3 dys.
144	1-15 hrs.	34 hrs.	223	6½ hrs.	73 hrs.	179	94 min.	3 dys.
145	17 hrs.	31 hrs.	224	1-16 hrs.	75 hrs.	190	3 hrs.	3 dys.
146	18 hrs.	24 hrs.	225	1-16 hrs.	80 hrs.	191	7 hrs.	3 dys.
147	1-15 hrs.	40 hrs.						
Average	10 hrs.	32 hrs.	Average	8-16 hrs.	77 hrs.	Average	2 hrs.	3 dys.

Tempera- ture.	No. of ex- periments.	Tetanus in.	Tempera- ture.	No. of ex- periments	Tetanus in.	Tempera- ture.	No. of ex- periments.	Tetanus in.
13° C.	10	0	13° C.	10	8	13° C.	20	20
			No. of frog.	Before onset.	Duration.	No. of frog.	Before onset.	Duration.
			80	4 hrs.	2-15 hrs.	90	130 min.	—
			98	2½ hrs.	18 hrs.	91	130 min.	4-22 hrs.
			99	2½ hrs.	4-18 hrs.	92	123 min.	1-15 hrs.
			107	3½ hrs.	4-16 hrs.	93	67 min.	3-20 hrs.
			109	3 hrs.	4-16 hrs.	156	163 min.	4-20 hrs.
			247	3 hrs.	—	157	135 min.	4-20 hrs.
			248	3 hrs.	—	158	194 min.	4-20 hrs.
			249	3 hrs.	—	159	192 min.	4-20 hrs.
						235	136 min.	—
						236	124 min.	—
						237	132 min.	—
						265	22 min.	—
						266	40 min.	—
						267	38 min.	—
						270	89 min.	—
						271	88 min.	—
						286	70 min.	—
						287	88 min.	—
						288	197 min.	—
						289	85 min.	—
			Average	3 hrs.	4-16 hrs.	Average	105 min.	4-15 hrs.

TABLE I.—*Concluded.*

Dose 0.0003 mg. per gm.			Dose 0.0005 mg. per gm.			Dose 0.0006 mg. per gm.		
Tempera- ture.	No. of ex- periments.	Tetanus in.	Tempera- ture.	No. of ex- periments.	Tetanus in.	Tempera- ture.	No. of ex- periments.	Tetanus in.
18° C.	6	2				16°-18° C.	7	7
No. of frog.	Before onset.	Duration.	No. of frog.	Before onset.	Duration.	No. of frog.	Before onset.	Duration.
118	50 min.	2½ hrs.	176	160 min.	—	176	160 min.	—
119	124 min.	1½ hrs.	177	180 min.	—	177	180 min.	—
			186	85 min.	3 hrs.	186	85 min.	3 hrs.
			187	148 min.	2 hrs.	187	148 min.	2 hrs.
			241	54 min.	—	241	54 min.	—
			242	85 min.	—	242	85 min.	—
			243	80 min.	—	243	80 min.	—
Average	87 min.	2 hrs.	Average	113 min.	2½ hrs.	Average	113 min.	2½ hrs.

Tempera- ture.	No. of ex- periments.	Tetanus in.	Tempera- ture.	No. of ex- periments.	Tetanus in.	Tempera- ture.	No. of ex- periments.	Tetanus in.
21°-24° C.	11	2	21°-24° C.	10	2	21°-24° C.	8	8
No. of frog.	Before onset.	Duration.	No. of frog.	Before onset.	Duration.	No. of frog.	Before onset.	Duration.
72	16 min.	6 hrs.	194	120 min.	½ hr.	94	35 min.	4 hrs.
73	14 min.	5½ hrs.	195	96 min.	1 hr.	95	110 min.	5 hrs.
						96	23 min.	2 hrs.
						97	31 min.	2 hrs.
						174	34 min.	3 hrs.
						175	32 min.	4½ hrs.
						212	48 min.	1 hr.
						213	57 min.	3 hrs.
Average	15 min.	5½ hrs.	Average	108 min.	¾ hr.	Average	46 min.	3 hrs.

Tempera- ture.	No. of ex- periments.	Tetanus in.	Tempera- ture.	No. of ex- periments.	Tetanus in.	Tempera- ture.	No. of ex- periments.	Tetanus in.
27°-30° C.	12	4	27°-30° C.	16	16	27°-30° C.	4	4
No. of frog.	Before onset.	Duration.	No. of frog.	Before onset.	Duration.	No. of frog.	Before onset.	Duration.
78	22 min.	4 hrs.	110	51 min.	5½ hrs.	218	24 min.	3½ hrs.
79	35 min.	4 hrs.	111	57 min.	1½ hrs.	219	53 min.	3 hrs.
130	45 min.	½ hr.	112	42 min.	3½ hrs.	220	62 min.	2½ hrs.
131	40 min.	2 hrs.	113	40 min.	2½ hrs.	221	60 min.	3½ hrs.
			116	21 min.	1½ hrs.			
			117	17 min.	—			
			120	25 min.	—			
			121	36 min.	—			
			128	35 min.	2 hrs.			
			129	29 min.	½ hr.			
			138	30 min.	2½ hrs.			
			139	58 min.	1½ hrs.			
			140	23 min.	1 hr.			
			141	50 min.	½ hr.			
			142	33 min.	2 hrs.			
			143	27 min.	2 hrs.			
Average	35 min.	2½ hrs.	Average	36 min.	2 hrs.	Average	69 min.	3 hrs.

there is no response and the legs if passively flexed are not extended until another attack of tetanus occurs.

DISCUSSION AND SUMMARY.

In order to establish the influence of temperature upon the effect of varying doses of strychnin injected into frogs, the animals must be kept under observation for several days and at various definite degrees of temperature. Statements that the animal was kept "cold," "at room temperature," or "warm" are insufficient. With a certain dose tetanus may result constantly at 30° C. yet never appear at 21° C., and either of these temperatures might be described as warm, when compared to a room temperature of 15° C. Furthermore an animal may apparently fail to respond in the cold to an injection of certain doses of strychnin and yet be found in tetanic convulsions the next day. That an animal may have late, long lasting, or strong tetanus while kept at such a low temperature as 5° C. after an injection of a dose of strychnin smaller than 0.01 of a milligram per frog emphasizes the fact that great caution must be exercised in formulating laws as to the influence of temperature on drug action.

The main results of this investigation may be summarized as follows: Doses of strychnin amounting to 0.0006 of a milligram per gram of frog will cause tetanus at all temperatures between 5° C. and 30° C., although at low temperatures the tetanus may appear late. A dose of 0.0003 of a milligram per gram of frog will frequently produce tetanus at 5° C. as well as at 30° or 27° C., but may nevertheless fail to produce any reaction at such an intermediary temperature as 21° C. Smaller doses, 0.0002 of a milligram per gram, will cause tetanus in the cold but not at high temperatures.

It may be stated in general that in frogs kept at low temperatures the tetanic state sets in later, continues longer, and each tetanic attack is of longer duration, while in the interval between the attacks the state of tonus is higher and the animals are more irritable than when they are kept at higher temperatures.

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ON ELIMINATION THROUGH THE MUCOSA OF THE URINARY BLADDER.*

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In a previous communication (1) it was reported that after intravenous injections of large amounts of dextrose into normal rabbits the intestine contained on the average 1.2 per cent. of the injected dextrose, while through the kidneys about 40 per cent. of the injected dextrose was eliminated. In rabbits whose kidneys were removed previous to the injection of the dextrose, and in which, therefore, nearly one half of the injected quantity was prevented from being eliminated, the dextrose content of the intestine reached the average of 2.2 per cent. In discussing these results we arrived at the conclusion that the elimination of 1.2 per cent. of the dextrose in the intestines of normal rabbits can not be looked upon as an "intestinal diabetes" (MacCallum (2)), nor is it due to an increased permeability of the intestinal epithelium (Fischer (3)), nor can the increase of 1 per cent. in the dextrose in the intestine of nephrectomized rabbits be looked upon as a "vicarious action" (MacCallum). It was assumed that the elimination of 1.2 per cent. in the intestine of the normal, and 2.2 per cent. in the intestine of the nephrectomized rabbit after an intravenous injection of a large quantity of dextrose has no specific secretory character, comparable, for instance, with the elimination of dextrose through the kidney. It was suggested that the intestinal elimination is merely due to the temporary hyperglycemia; and, in consequence of the high sugar content of the blood and lymph, elimination occurs in minute quantities through all mucous membranes. In the intestine, on account of the large surface of its mucosa, the sugar eliminated aggregates to the considerable figures of 1.2 and 2.2 per cent.

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The mucosa of the intestine, however, is studded with numerous glands. While each kind of gland secretes a specific substance, all glands eliminate some fluid in which these substances are suspended. The glands derive that fluid from the blood. It is therefore plausible to assume that in taking out some fluid from the blood the glands may be capable of removing from the blood, in very minute quantities and in an unspecific manner, any substance present in the blood in excess of the normal. All mucous membranes are moist; their moisture they also derive from the lymph and the blood. Are mucous membranes as such, without the assistance of glands, also capable of eliminating from the blood substances present in the blood in excess? To test this question, one must examine the contents of a viscus which is lined with a mucous membrane free from glands. Such a hollow viscus is the urinary bladder; its mucosa is not provided with secreting glands. A series of experiments was therefore carried out to determine whether dextrose, when present in excess in the blood, or a substance foreign to the blood, may be eliminated into the bladder. In the following report we shall describe these experiments very briefly.

The rabbit was the experimental animal used. A double nephrectomy was performed and either later on the same day, or on the next day, cannulas were inserted in the bladder and in the jugular vein. Ether anesthesia was used during all operative procedures. In the bladder was inserted a cannula which was modeled on the Ludwig-Spengler cannula for obtaining blood pressure laterally from an artery, except that both plates were made flat. Care was taken, when inserting the cannula, that the circulation was interfered with as little as possible. The cannula was inserted in the ventral side of the bladder and the abdomen sewed up. Warm 20 or 30 per cent. dextrose solution was injected intravenously, seven to ten grams per kilogram being introduced in from twenty to fifty minutes. The bladder was washed by irrigating with warm 0.9 per cent. sodium chloride several times during and after the dextrose injection; the fluid was ejected by the animal as in normal micturition. The washings were boiled immediately, assembled, and concentrated to a small volume on the water bath and analyzed for sugar, qualitatively with Fehling's and Benedict's (4) solutions, and quanti-

TABLE I.

No. of animal.	Color.	Sex.	Weight in gm.	Time of nephrectomy.	Uranin dissolved in mg.	Dextrose introduced.			Duration of dextrose injection in min.	First bladder washing.	Color of bladder washings.	Uranin found in bladder washings.	Dextrose found in bladder washings.
DB 2	Gray	F.	1,550	Previous day	0	54	20	7	34	After injection of 22 c.c. dextrose solution	Blood-tinged		0.006
DB 3	Gray	M.	2,400	Same day	0	84	20	7	29	After 42 c.c. dextrose solution	Slightly blood-tinged		0.000
DB 5	Gray	M.	2,020	Previous day	0	70	20	7	20	After 57 c.c. dextrose solution	Colorless		0.000(?)
DB 6	Gray	F.	2,030	Previous day	0	70	20	7	33	After 35 c.c. dextrose solution	Almost colorless		0.007
DB 7	Maltese and white	F.	1,440	Same day	0	50	20 ¹	7	20	After 25 c.c. dextrose solution	Almost colorless to slightly blood-tinged		0.008
DB 11	Gray and white	M.	1,770	Same day ²	0	62	20 ²	7	26	After 31 c.c. dextrose solution	Almost colorless		0.000
DB 12	Gray	M.	2,055	Previous day	0	72	20	7	34	1 hour after end of dextrose and thereafter for 1 hour	Colorless to slightly blood-tinged		0.000
DB 13	Maltese and gray	F.	1,420	Previous day	0	50	20 ⁴	7	31	After 25 c.c. dextrose solution	First very bloody, others almost colorless		0.000
DB 14	Gray	F.	1,740	Same day	20	58	30	10	48	After 5.5 c.c. dextrose solution	Almost colorless	0	0.000(?)
DB 15	Gray	F.	2,140	Same day	100	72.5	30	10	40	After 18 c.c. dextrose solution	Almost colorless to bloody	Trace(?)	0.000(?)
DB 17	Gray	M.	1,625	Same day	100	55	30	10	38	After 11 c.c. dextrose solution	Colorless	0	0.000
DB 18	Gray	M.	1,470	Same day	100	52	30	10	35	After 13 c.c. dextrose solution	Colorless to fluorescent	<0.035 mg.	0.011
DB 19	Gray	M.	1,550	Same day	100	52	30	10	40	After 13.5 c.c. dextrose solution	Colorless to fluorescent	<0.15 mg.	0.000

¹ 50 c.c. $\frac{M}{8}$ magnesium chloride first injected in 21 minutes. Artificial respiration after 18 c.c. had been introduced.

² Only ureters tied.

³ 150 c.c. 0.9 per cent. sodium chloride first injected in 38 minutes.

⁴ 50 c.c. $\frac{M}{8}$ calcium chloride first injected in 34 minutes.

tatively by the Pavy-Ramsden method (5). In some of the experiments uranin, the sodium salt of fluorescēin, in very diffusible dye, was dissolved in the injection fluid. This was estimated in the washings by a rough colorimetric analysis.

The results of all the experiments are given in table I. It will be seen that in nine experiments either no sugar or else indeterminable traces were found, and in four experiments six to eleven milligrams, *i. e.*, 0.05 to 0.08 per cent. of the amount injected, were obtained. In three experiments the injection of magnesium chloride, sodium chloride, and calcium chloride, respectively, in physiological concentration, had no noticeable effect upon the result. In the experiments in which twenty milligrams of uranin were introduced, no trace of the dye could be detected in the washings of the bladder. In four experiments 100 milligrams of uranin were introduced; in two there was not a trace and in the other two there was a barely perceptible trace.

In considering these results stress is to be laid on the following facts: (1) the amount of sugar (and uranin) injected was exceedingly great; (2) the kidneys were not functioning; and (3) the bladder was washed at frequent intervals. Nevertheless either there was no dextrose or uranin discovered in the washings or the amounts discovered were quite insignificant.

The conclusion may therefore be drawn that the bladder is practically impermeable for diffusible substances present in the blood in great excess even in the absence of the kidneys, the chief organs of elimination of the body.

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AN ELECTROCARDIOGRAPHIC STUDY OF THE ANAPHYLACTIC RABBIT.*

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PLATES 33 TO 37.

INTRODUCTION.

The subject of cardiac disturbances in serum anaphylaxis has not received much attention from investigators. Gay and Southard,¹ in their extensive histological studies, noted that the heart of the anaphylactic guinea pig often shows hemorrhages; they did not, however, observe any alteration in the functional activity of this organ, as far as we are aware. The first definite observation of a pathological alteration of the heart's activity during serum anaphylaxis was made by Auer and Lewis,² who observed that the heart of a guinea pig dying from anaphylaxis showed several abrupt diminutions in rate, and that these rates were in simple ratio to each other. This observation was made by inspection of the exposed heart and by examination of graphic records. The block was thought to be due to asphyxia. This interpretation is in accord with the results Sherrington³ obtained with decapitated, atropinized cats, where asphyxia produced an abrupt halving of the cardiac rate; it also agrees with the work of Lewis and Mathison,⁴ who demonstrated the occurrence of heart-block in cats during asphyxia by means of the electrocardiograph.

During the anaphylactic reaction of the rabbit, one of us⁵ has noticed heart-block, both by inspection of the exposed heart and by a graphic method. Further and more detailed evidence will be given in this paper.

Cardiac disturbances during serum anaphylaxis of the cat have been described by Schultz,⁶ but his results are difficult to interpret with certainty. In the cat matters are complicated by the fact that horse serum exerts a strongly toxic effect in the unsensitized animal; as little as 0.25 c.c. per kilo usually kills young

* A preliminary note was published in the *Zentralblatt für Physiologie*, 1913, xxvii, 1. Received for publication, July 1, 1913.

¹ Gay, F. P., and Southard, E. E., *Jour. Med. Research*, 1907, xvi, 146, 176.

² Auer, J., and Lewis, P. A., *Jour. Exper. Med.*, 1910, xii, 171; see plate IX, figure 5, for a graphic record.

³ Sherrington, C. S., *Jour. Physiol.*, 1909, xxxviii, 381.

⁴ Lewis, T., and Mathison, G. C., *Heart*, 1910, ii, 47.

⁵ Auer, J., *Jour. Exper. Med.*, 1911, xiv, 483 (see plate 44 and plate 47, figure 4).

⁶ Schultz, W. H., *Jour. Pharmacol. and Exper. Therap.*, 1911-12, iii, 302.

cats, while adult cats are strongly affected but "frequently recover" from this dose.⁷ Even 0.1 c.c. per kilo gives a fall in blood pressure both in normal and sensitized cats.⁸ The ordinary reinjection dose employed by Schultz seems to have been 0.2 c.c. per kilo. Moreover, Schultz observed traces of blood clot in the hearts of some anaphylactic cats.⁹ The cat thus differs strikingly from guinea pigs, rabbits, and dogs in its reaction to horse serum, for the latter animals show no definite toxic effects when comparatively large quantities of not too fresh horse serum are injected intravenously. A normal guinea pig tolerates 5 c.c., a normal rabbit 15 c.c., and a normal dog at least 30 c.c. of horse serum intravenously without any symptoms which even remotely resemble those occurring in anaphylaxis.¹⁰ A consideration of these data forces us to the conclusion that the cardiac disturbances noted by Schultz are not necessarily anaphylactic phenomena.

The anaphylactic dog also shows heart-block and other cardiac disturbances demonstrable by the electrocardiograph. A detailed statement of these changes will be found in another paper.¹¹

This brief review of the literature concerning heart disturbances in serum anaphylaxis of the intact animal shows definitely that alterations of the functional activity of this organ are common in this state and apparently form an integral part of anaphylaxis. In the following pages we shall give an account of the main changes which the heart of the anaphylactic rabbit reveals when examined by the electrocardiograph. Details regarding the process of sensitization, which is by no means as certain in the rabbit as in the guinea pig, will be found in a former paper by one of us.

EXPERIMENTAL PART.

The cardiac changes of anaphylactic reaction were studied in twenty-four rabbits. Young animals weighing about one kilo were sensitized by repeated subcutaneous injections of horse serum. About four weeks after the last injection, the animals were given the final, toxic dose of about 9 c.c. into a marginal ear vein, and the cardiac disturbances were studied by means of electrocardiograms taken at short intervals immediately before, during, and after the injection. The records were made with the large Edelmann string galvanometer. The animals were connected with the instrument by electrodes

⁷ Schultz, W. H., *loc. cit.*, p. 300.

⁸ Schultz, W. H., *loc. cit.*, p. 317.

⁹ Schultz, W. H., *loc. cit.*, p. 304.

¹⁰ These doses do not represent the maximal quantities which are tolerated; they are quoted because such doses have been injected in normal animals during the course of other experiments.

¹¹ Robinson, G. C., and Auer, J., *Zentralbl. f. Physiol.*, 1913, xxvii, 383; *Jour. Exper. Med.*, 1913, xviii, (in press).

usually attached to the right fore leg and left hind leg (the second lead). The string of the galvanometer was arranged so that a deviation of 1 cm. in the curve represents the passage of 1 millivolt. No ether was given during the experiment, and the slight operative procedures hardly demanded it. In eleven of the sensitized rabbits both vagi were cut before the toxic dose of serum was injected and after tracheotomy had been performed. Horse serum was also injected intravenously into two non-sensitized rabbits, from which electrocardiographic records were obtained in the same way as from the others.

The rate of the heart-beat, the relation of auricular to ventricular contractions, and the form of the electrocardiograms were especially considered in studying the records obtained in these experiments. Definite abnormalities in the heart-beat were observed in twenty-two of the twenty-four experiments in which sensitized rabbits were used, and in ten experiments the anaphylactic reaction was fatal, the rabbits dying in from two to nineteen minutes. The average time of death was seven minutes after the beginning of the intravenous injection. In these experiments it is necessary to separate the cardiac disturbances which resulted primarily from the anaphylactic reaction, from those which occurred as phenomena of the dying heart, and cessation of respiration was therefore used as the index of death. Changes in the heart-beat, which occurred after respiration had ceased, were not considered as phenomena of the anaphylactic reaction, although in some instances the electrocardiograms returned to normal after respiration had ceased, while they had been markedly abnormal before.

The intravenous injection of horse serum into two non-sensitized normal rabbits was followed by no such changes in the electrocardiograms as were seen in the sensitized animals. In one control experiment the records indicated that premature ectopic ventricular beats began to occur occasionally about thirty seconds after the beginning of the injection of horse serum and before it was completed. The ectopic beats became more numerous, and one minute after the beginning of the injection they alternated with the normal beats. These ectopic ventricular contractions gradually became less numerous, and in three and a half minutes after the beginning of the injections the records show that they had ceased to occur. Three days later this rabbit was again attached to the string galvanometer and given an intravenous injection of nine cubic centimeters of

normal saline solution. No disturbances of the cardiac mechanism occurred. In the second control experiment the records show that no cardiac disturbances followed the intravenous injection of horse serum.

A second intravenous injection of horse serum was given to seven sensitized rabbits after the effects of the first injection had passed off. All but one of the rabbits in these experiments had shown marked cardiac disturbances after the first injection. After the second injection no disturbances of the heart-beat or other symptoms occurred except in one experiment in which ectopic ventricular contractions appeared. These animals were thus in the anti-anaphylactic state at this time. The fact that ectopic ventricular contractions occurred after the second injection in one of these animals and also in one of the non-sensitized animals after a single injection of horse serum, indicates that the occurrence of such beats probably cannot be considered as significant when they occur during the anaphylactic reaction.

The condition of the various experiments and the results may be summarized briefly as follows:

No. of experiment.	Result.	Vagi.	Complex changes before cessation of respiration.
2	Fatal	Intact	Marked. Temporary partial block. Slight changes in form.
3	Fatal	Intact	Marked. Temporary partial block. T wave much increased.
5	Fatal	Intact	Marked. Abnormal relation of P and R waves. T wave disappeared.
6	Fatal	Intact	Marked. Abnormal relation of P and R waves. S wave developed.
7	Fatal	Intact	Moderate. Curves poor. T waves disappear. Ectopic beats.
8	Fatal	Intact	Marked. Temporary block twice. Marked S and negative T waves developed.
10	Fatal	Cut	Marked. S wave and negative T wave developed.
17	Fatal	Cut	Marked. P waves disappear. Possible auricular fibrillation.
19	Fatal	Cut	Marked. T partly fused with R waves.
25	Fatal	Cut	Marked. P wave disappears. T close to R waves.
1	Not fatal	Intact	Moderate. P wave disappears. T wave becomes absent.
4	Not fatal	Intact	No definite changes.
9	Not fatal	Intact	Marked. Abnormal relation of P and R waves. T wave disappears.
11	Not fatal	Intact	Marked. Abnormal relation of P and R waves. Large S and negative T waves.

No. of experiment.	Result.	Vagi.	Complex changes before cessation of respiration.
12	Not fatal	Intact	Marked. Abnormal relation of P and R waves. Abnormal R. S develops.
13	Not fatal	Intact	Slight. S wave develops. T wave becomes negative.
14	Not fatal	Intact	Slight. S wave develops. T wave disappears.
18	Not fatal	Cut	Marked. Prominent S wave develops and large T waves.
20	Not fatal	Cut	Marked. Abnormal relation of P and R waves. Ventricular complexes vary.
21	Not fatal	Cut	Marked. R waves broad. T waves negative.
22	Not fatal	Cut	Marked. Broad, deep S wave.
23	Not fatal	Cut	Moderate. S wave develops. T wave disappears.
24	Not fatal	Cut	Marked. Abnormal relation of P and R waves. P wave disappears. Broad S wave.
26	Not fatal	Cut	No definite changes.
15	Control	Intact	No changes except ectopic ventricular beats.
16	Control	Intact	No definite changes.

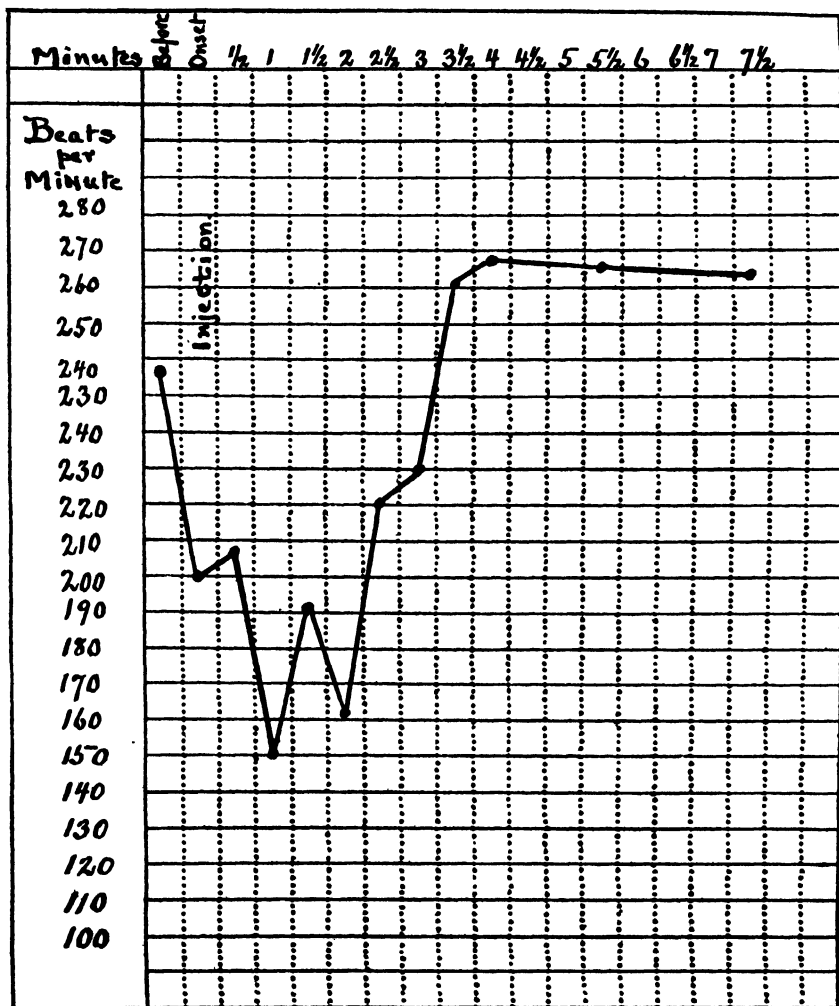
The experiments may be grouped into those in which the anaphylactic reaction was fatal, and those in which the animals recovered. These groups may be subdivided according to whether the vagi were intact or cut.

The changes in cardiac rate may be considered, however, independent of these groups, for seventeen of the twenty-four experiments showed an alteration of rate which bore apparently no relation to the ultimate outcome of the experiments, nor to whether the vagi were intact or not. This change consisted of a slowing of twenty-five beats or more during the first minute after beginning the injection, and then an increase to a rate twenty-five beats or more per minute faster than that present before the injection. This change of rate may be illustrated by a chart constructed from experiment 12, a non-fatal experiment with the vagi intact (text-figure 1). The same initial slowing of the heart is shown in blood pressure records.¹²

The first group consists of six experiments in which the final injection of horse serum was fatal and in which the vagi were intact. In all but one of these cases marked disturbances of the heart-beat developed swiftly, often before the end of the serum injection, and at a time when the respiration was practically undisturbed. The

¹² Auer, J., *Jour. Exper. Med.*, *loc cit.*, p. 495 (plates 44, 45, and plate 47, figure 4).

most striking disturbance was partial heart-block, which occurred in three of the cases. This condition was always temporary, and the normal sequential beat was established before the changes of the dying heart occurred. In experiment 8 partial heart-block occurred one and a half minutes after the beginning of the injection (figures 1 and 2). The normal sequential beat returned forty-five



TEXT-FIG. 1. Experiment 12. The changes in the cardiac rate in anaphylactic shock.

seconds later with an increase in rate and a moderate change in the form of the complexes (figure 3). Partial heart-block appeared again in one and a quarter minutes, when the complexes had changed markedly in form (figure 4). The block had disappeared five minutes after the beginning of injection (figure 5), but returned again as a phenomenon of the dying heart in less than one minute (figures 6 and 7). In experiment 3 (figure 8) a partial heart-block was present one minute and a half after the injection (figure 9), while in three minutes complete dissociation of auricles and ventricles developed (figure 10). The sequential beat had returned at four minutes after the injection, but with a marked delay in the conduction time (figure 11). Half a minute later partial block had again set in (figure 12). The various electrocardiograms obtained from this animal showed marked changes in form, and the record obtained shortly after the injection shows ventricular complexes very much of the same form as those taken eleven minutes after the injection and one minute after respiration had ceased (figure 13). In each, the sharp, rather prominent T waves are close to the R waves, the down strokes of which are slow and do not reach the base line. This form of ventricular complex seems to be characteristic of the dying heart, as shown by one of us (Robinson).¹³ In experiment 2 partial heart-block was present from two to four and a half minutes after the injection and returned again after the normal sequential beat had been present for half a minute, continuing until respiration ceased ten minutes after the injection.

In two experiments an abnormal relation between the P and R waves occurred in the electrocardiograms. This consisted of a temporary shortening of the conduction (P-R) time. Thus in experiment 6 the P-R time was 0.08 of a second before the injection (figure 14), while it became reduced to 0.033 of a second two minutes after the injection was begun (figure 15). In half a minute the P-R time had lengthened to 0.068 of a second. In experiment 5 the P-R time was diminished at forty-five seconds after the onset of the injection, and remained so for one and a quarter minutes. The P-R relation was then normal for one minute, when

¹³ Robinson, G. C., *Jour. Exper. Med.*, 1912, xvi, 291.

the P and R waves were again abnormally near each other and partially fused. This same change in the relation of the P and R waves occurred in the electrocardiograms from five other experiments in which the anaphylactic shock was not fatal, three in which the vagi were intact, and two in which they had been cut.

In the four experiments in which a fatal anaphylactic shock occurred after the vagi had been cut, definite abnormalities in the forms of the electrocardiograms appeared in forty-five seconds, one and a half minutes, two minutes, and two and a half minutes respectively after the beginning of the injection. In one experiment respiration ceased at about the same time that the electrocardiograms became abnormal, but in the others intervals of two, four, and eight minutes respectively separated the appearance of abnormalities in the electrocardiograms and the cessation of respiration. The changes in form of the electrocardiograms from these experiments were various. In two the P wave disappeared, and in one of these the electrocardiograms suggest indefinitely that auricular fibrillation was present for a short time. The T waves of ventricular complexes changed definitely in three of the experiments, in two occurring close to or partly fused with the R wave. In the other experiment which showed abnormal T waves, they gradually diminished in size and then became definitely negative or downwardly directed, this change beginning two and a half minutes after beginning the injection and reaching its maximum one and a half minutes later (figures 16 and 17). Thus it is seen that changes occurred in the electrocardiograms from this group of experiments which indicate that both the auricular and the ventricular activity were disturbed, while no definite disturbances of conduction took place.

The experiments in which the anaphylactic shock was not fatal, are conveniently divided into those in which the vagi were intact and those in which the vagi were cut. There were seven experiments with intact vagi, and the electrocardiograms from all but one showed changes which were marked in three, moderate in one, and slight in two. The abnormalities in the electrocardiograms were first seen in from one to three and a half minutes after the beginning of the injection. In all these experiments but one, the electro-

cardiograms returned to the form seen before the injection, the time varying from seven and a half to twenty-one minutes. These changes vary in the different experiments. Those which occurred in experiment 11 are shown in figures 18 to 21; before injection the complexes are well formed, the P-R time is 0.07 and the rate 281; three and a quarter minutes later the R wave is followed by a negative phase, the P-R time is unchanged, while the rate has decreased to 174 beats per minute; fifteen seconds after this a marked arrhythmia with varying electrocardiograms set in, which disappeared in another fifteen seconds, when very abnormal electrocardiograms occurred. This form, consisting mostly of a broad negative wave, persisted until five and a half minutes after the injection, when it began to return gradually to the form seen before the injection.

The T waves were changed in four of these seven experiments, disappearing in three, and in one becoming downwardly directed. The P and R waves had an abnormal relation, temporarily, in three experiments, becoming approximated as in experiment 6 (figures 14 and 15), and in one experiment the P wave disappeared.

Of the seven non-fatal experiments in which the vagi were cut, five showed marked changes in the form of the electrocardiograms; in one there were moderate changes, and in one no changes were seen. The changes appeared after one to five minutes, and the abnormalities lasted from two and a half to five minutes. The changes in this group resemble those which occurred in the non-fatal experiments where the vagi were not cut. In four experiments large, broad, downwardly directed S waves appeared. The T wave changed in three, once increasing in size, once disappearing, and once becoming downwardly directed. In two experiments the P approximated the R waves, and in one the P waves disappeared. An example of the marked changes which occurred in the electrocardiograms of this group of cases is given in figures 22 to 24.

DISCUSSION.

The experiments which have been described show that in the anaphylactic reaction produced by intravenous injections of horse serum into sensitized rabbits, disturbances of the heart occur in a

large majority of cases. These disturbances are seen in non-fatal as well as in fatal anaphylaxis, and after the vagi are cut as well as when they are intact. There were but two negative experiments in twenty-four, and in these the rabbits showed no signs of anaphylaxis. It is thus evident that cardiac disturbances are practically constantly present in rabbits during the anaphylactic reaction, in contrast to dogs, where changes in the heart-beat were noted in but six of twelve experiments,¹⁴ although a marked fall of blood pressure was constant. An intravenous injection of horse serum does not cause such cardiac disturbances in non-sensitized rabbits, nor in rabbits in the anti-anaphylactic state.

The cardiac disturbances vary considerably. They may consist of disturbances in conduction, giving rise to partial heart-block, which occasionally goes on to complete dissociation of auricles and ventricles. Partial heart-block occurred in our series only in experiments in which there was fatal anaphylaxis and in which the vagi were intact. The number of experiments is too small, however, to indicate whether or not it is only under such conditions that partial heart-block may develop. That the relationship between auricular and ventricular activities may become disturbed is indicated by the abnormal proximity of the P and R waves (figure 15). This change, which was seen only once in the series on dogs, occurred in seven experiments of this series. Its significance has been discussed in our paper on cardiac anaphylaxis in the dog; it probably represents a change in the position of the point at which the stimulus of the heart-beat arises.

The changes in form of the electrocardiograms, especially of the portions representing ventricular systole, are various. The waves most frequently altered are the T waves, which disappeared in six experiments, became negative or downwardly directed in five, and increased in size in four. In eleven experiments, negative or downwardly directed S waves developed during the anaphylactic reaction. The changes in the form of the electrocardiograms suggest damage to the ventricles, which renders their contractions abnormal. A discussion of the various forms encountered is not in the scope of this paper. It may be said, however, that changes occur similar

¹⁴ Robinson, G. C., and Auer, J., *loc. cit.*

to those obtained by Eppinger and Rothberger¹⁵ following the injections of toxic substances directly into the ventricular musculature of the dog.

The cardiac disturbances in sensitized rabbits occur very soon after the intravenous injections of serum, in some experiments before the end of the injection. They were usually observed from thirty seconds to three minutes after the beginning of the injection, although in a few experiments five minutes elapsed before cardiac disturbances occurred.

The heart apparently recovers quickly in the non-fatal experiments, and electrocardiograms similar to those obtained before the injection appeared from two and a half to eighteen minutes after the abnormalities set in. The oscillation between normal and abnormal forms of the electrocardiograms was usually gradual, but in several experiments marked abnormalities occurred or disappeared suddenly. In the fatal cases it was also noted that early occurring abnormalities tended to disappear before the changes of the dying heart set in. This was seen especially well in those experiments where partial heart-block occurred, for the normal sequential beat was usually reestablished once or even twice before the final changes of cardiac death appeared.

SUMMARY.

Electrocardiographic examination of rabbits during the anaphylactic reaction revealed marked and various changes of the heart's activity in twenty-two out of twenty-four animals. Changes occurred in fatal as well as in non-fatal cases, after the vagi were cut as well as when they were intact. Cardiac disturbances are thus a practically constant result of serum anaphylaxis in the rabbit. It therefore is possible that anaphylaxis plays a rôle in the causation of certain cardiac derangements in man.

¹⁵ Eppinger, H., and Rothberger, C. J., *Wien. klin. Wchnschr.*, 1909, xxii, 1091.

THE CHEMISTRY OF GLUCONEOGENESIS.

III. THE FATE OF ISOBUTYRIC, ISOVALERIANIC AND ISOCAPROIC ACIDS IN THE DIABETIC ORGANISM, WITH CONSIDERA- TION OF THE INTERMEDIARY METABOLISM OF LEUCINE AND VALINE.*¹

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sylvania, Philadelphia.)

For the proper understanding of the processes involved in the metabolism of the different foodstuffs it is essential to know the fate of the individual products of metabolism in the body. For the past few years this question has been the center of attack from different angles. The difficulties associated with these researches appeared insurmountable at times, but with the latest developments in the application of organic chemistry to biological processes more and more light is being thrown on the subject of the intermediary metabolism of foodstuffs.

There are a number of methods that have been devised of late for the prosecution of these researches, and while it must be admitted that every one of them may be associated with a certain amount of error, they have nevertheless all helped to bring to light certain definite reactions. The entire subject has been recently reviewed² and much space need not be given here to this phase of the problem.

In these researches animals were made diabetic by daily injections of 1 gram of phlorhizin ground up in a mortar with 10 cc. of olive oil. The urine was collected by catheter and at the end of

* Received for publication, April 30, 1913.

¹ Aided by a grant from The Rockefeller Institute for Medical Research.

² Dakin: *Oxidations and Reductions in the Animal Body*, Longmans, Green and Company, London, 1912.

each period of twelve hours the bladder was washed three times with warm distilled water.

The urine was analyzed for its optical activity, nitrogen, glucose, ammonia, acetone, aceto-acetic acid and β -hydroxybutyric acid. In these determinations we were assisted by Mr. C. A. Hornberger.

The original purpose of these researches was to find the chemical configuration that a substance required in order to become converted into glucose in the diabetic organism. It soon became evident that the study of this problem was so closely interwoven with questions of the intermediary metabolism of foodstuffs and substances chemically related to them, that it was deemed necessary to extend the scope of this investigation to a broader field.

The method employed in these researches permits of the study of the following problems:

I. The fate of different substances in the animal body with reference to their ability to form glucose.

II. The fate of different substances with reference to their ability to give rise to β -hydroxybutyric acid, aceto-acetic acid and acetone.

III. The chemical changes that different substances undergo in the process of catabolism in the animal body before their conversion into glucose or acetone bodies.

IV. The influence of different substances on the metabolic processes of the diabetic organism—especially antiketogenesis.

V. The rôle of different substances in the pathology of diabetes.

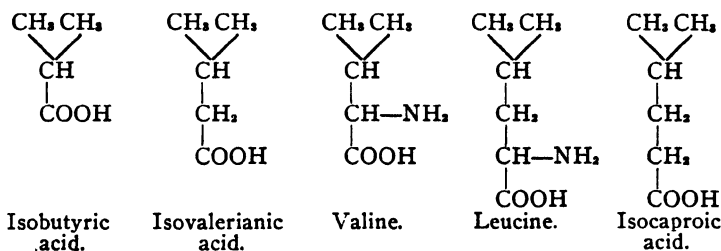
In the first two papers of this series it was shown that the normal fatty acids in the processes of catabolism in the diabetic animal undergo very definite reactions, by becoming oxidized in the β -position, giving rise to an acid with two carbons less. This conclusion was reached after having established the fact that propionic acid (when fed to diabetic dogs) is completely converted into glucose, and that of the higher fatty acids only those give rise to glucose which stand in relationship to propionic acid by having two (or a multiple thereof) carbons more. Thus *n*-valerianic and *n*-heptylic acids, containing five and seven carbons respectively, give rise to glucose, whereas *n*-butyric and *n*-caproic acids, containing four and six carbons respectively, do not give rise to glucose, but

to an increase in the acetone bodies. These findings add increased support to Knoop's hypothesis of β -oxidation.

In this communication are recorded the results of experiments with isobutyric, isovalerianic and isocaproic acids fed to diabetic dogs.

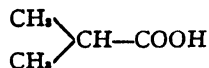
The methods employed were essentially the same as those of the previous experiments, with the exception that the phlorhizin was administered according to the Coolen method. It yields very satisfactory results, and with greater comfort to both operator and animal. The β -hydroxybutyric acid was determined by Shaffer's method.

The iso compounds enumerated above stand in very close relationship to several important amino-acids,



and the results of our experiments seem to throw light on the intermediary metabolism of these amino-acids.

THE EFFECT OF ISOBUTYRIC ACID.



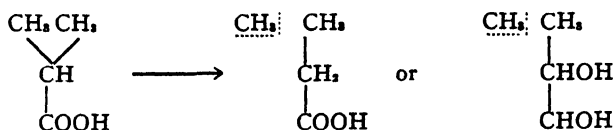
Baer and Blum³ gave 20 grams of isobutyric acid to a diabetic patient with the object of ascertaining whether it would cause increased elimination of the acetone bodies. They did not get any increase in the β -hydroxybutyric acid or acetone. Embden, Salomon and Schmidt⁴ perfused the liver with blood containing isobutyric acid and found no increase in the acetone formation.

In this series of experiments isobutyric acid was fed with a

³ Baer and Blum: *Arch. f. exp. Path. u. Pharm.*, lv, p. 89, 1906.

⁴ Embden, Salomon and Schmidt: *Hofmeister's Beiträge*, viii, p. 129.

double object in view. First, both Baer and Blum and Embden suggested the possibility of demethylation in the catabolism of the isobutyric acid molecule, with the introduction of either an H or an OH in the α -carbon:



If this were true, isobutyric acid would give rise to propionic acid or lactic acid, either of which should give rise to extra glucose. Second, it was of great importance to study the possible effects of isobutyric acid on acidosis.

In experiment XI, period VI, the animal was given subcutaneously 10 grams of isobutyric acid as sodium salt. The glucose elimination rose from 11.5 to 14.03 grams. The nitrogen elimination sank from 3.6 in the fore period to 3.29 and 2.55 in periods VI and VII, respectively, to rise again to 3.61 in period VIII. The amount of extra glucose eliminated was 5.52 grams (calculated by the method suggested by Lusk and Ringer).⁵ The most remarkable effect of the isobutyric acid was on the acetone and β -hydroxybutyric acid elimination. In the fore period the acetone elimination was 0.223 gram; it dropped down to 0.059 and rose again to almost the original level in period VII. The β -hydroxybutyric acid, which stood at 1.1 grams for two successive periods, dropped to 0.238 gram and returned to 1.325 grams as the effect of the isobutyric acid wore off. In period XII of the same experiment a similar dose of isobutyric acid was administered to the same dog. The effects were essentially the same, except that the amount of extra glucose was less than in the first case.

In periods IX and XII of experiment XII the animal received 10 grams of isobutyric acid. The yield of extra glucose was 7.6 grams in period IX and 4.3 grams in period XII.

In period VI of experiment XII the animal received 20 grams of isobutyric acid, which resulted in the elimination of 9.15 grams of extra glucose. In period XII of the same experiment the animal

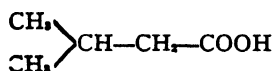
⁵ Ringer and Lusk: *Zeitschr. f. physiol. Chem.*, lxvi, p. 106, 1910.

was given subcutaneously 10 grams of isobutyl alcohol, which resulted in the elimination of 10.3 grams of extra glucose.

All these experiments prove beyond question that *isobutyric acid gives rise to considerable quantities of extra glucose*. In most of the cases there was a very marked diminution in the nitrogen output, immediately following the isobutyric acid administration. This phenomenon is very marked in experiments XI, XII and XIV. It is, however, absent in experiment XIII, periods VI and VII.

Another point of great interest is the effect of isobutyric acid on the elimination of acetone bodies. In experiment XI the effect was very marked, while in the others it was hardly noticeable. These individual differences point very strongly to the existence of certain factors in the metabolism of the diabetic organism which determine the action of isobutyric acid and similar substances. Since these experiments were performed, we have administered isobutyric acid to several patients, some with very severe and others with moderately severe acidosis. It was found that in severe cases of diabetes most of the isobutyric acid is converted into glucose, no effect whatsoever being exerted on the acidosis. In the milder cases it is similarly converted into glucose, but very distinct antiketogenetic effects are noted. The influence of isobutyric acid on the nitrogen metabolism in animals and on acidosis in human diabetes will form the subject of a separate communication in the near future.

THE EFFECT OF ISOVALERIANIC ACID.



That isovalerianic acid gives rise to large quantities of β -hydroxybutyric acid, aceto-acetic acid and acetone has been proven beyond question by Baer and Blum⁶ in the diabetic patient and by Embden⁷ who found an increase in the aceto-acetic acid formation on perfusing an excised liver with blood containing 2 grams of isovalerianic acid.

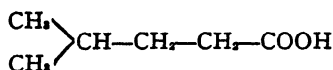
In this research it was our object to see whether isovalerianic

⁶ *Loc. cit.*

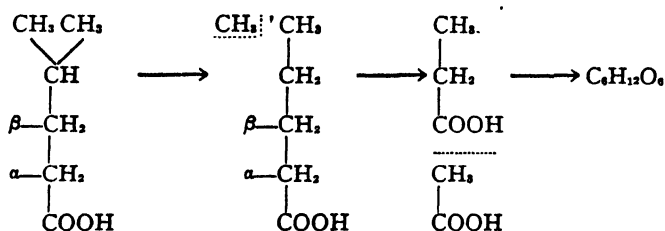
⁷ *Loc. cit.*

acid has any influence on the glucose elimination. In experiment XI period IX, in experiment XII period VI and in experiment XIV period XI, 10 grams of isovalerianic acid as sodium salt were administered subcutaneously. In none of these experiments was there any increase in the glucose. The elimination of the acetone bodies was increased to a remarkable extent, thus confirming the findings of Baer and Blum and of Embden.

THE EFFECT OF ISOCAPROIC ACID (ISOBUTYL ACETIC ACID).



Embden perfused a dog's liver with blood containing isocaproic acid and obtained no increase in the aceto-acetic acid formation. We performed our experiments with this substance to see what influence it has on acidosis and whether it will give rise to extra glucose. Reasoning *a priori*, one would expect this substance to give rise to glucose. The process of demethylation having been established in iso compounds in the case of isobutyric and isovalerianic acids, there is every reason to suppose that such a process takes place in the isocaproic acid molecule. Valerianic acid would be formed first, which after undergoing β -oxidation, would give rise to propionic acid and to glucose.



In period III of experiment XIII, 11.6 grams of isocaproic acid were given subcutaneously as sodium salt. The glucose elimination rose from 24.6 grams in the fore period to 25.19 grams, in spite of the drop in the nitrogen. The D:N ratio, which was 3.38 in the fore period and 3.36 in the after period, rose to 3.65. The extra glucose eliminated was 1.95 grams. In experiments XIV periods XVIII and XIX and in experiment XV period IX, 10

grams of isocaproic acid as sodium salt were given *per os*. The results in both cases corroborate the results in the first experiment in showing that extra glucose is formed from isocaproic acid. It is true that the amount of extra glucose does not come up to the theoretical value, but, as will be seen from other experiments, theoretical yields are not always obtained.

INTERMEDIARY METABOLISM OF FATTY ACIDS CONTAINING AN ISOPROPYL RADICAL.



From the work of Baer and Blum, Embden and our own, it is apparent that all compounds containing an isopropyl radical undergo demethylation. The question of interest in this connection is, in what way does the methyl radical dissociate from the rest of the molecule? Baer and Blum have advanced the suggestion that the CH_3 may leave the molecule and an OH radical take its place. They based this suggestion upon the fact that after feeding 20 grams of isobutyric acid they obtained 0.5 gram of zinc lactate from one-half of the twenty-four hours' urine, and also upon the fact that isovalerianic acid gave rise to β -hydroxybutyric acid. We are inclined to believe that the methyl radical is removed by a process of hydrolysis, the OH going to the methyl radical, forming CH_3OH , which is oxidized in the body, while the H radical goes to replace the CH_3 , forming an acid of the normal series. Thus isobutyric acid gives rise to propionic acid which in turn may give rise to lactic acid. Isovalerianic acid gives rise to butyric acid which in turn produces β -hydroxybutyric acid. This also explains why Baer and Blum obtained β -hydroxybutyric acid from α -methylbutyric acid and failed to get it from α -hydroxybutyric acid.

INTERMEDIARY METABOLISM OF AMINO-ACIDS CONTAINING AN ISOPROPYL RADICAL.

Embden and Marx⁸ in their perfusion experiments found that α -amino-valerianic acid gave rise to aceto-acetic acid, while neither

⁸ Embden and Marx: *Hofmeister's Beiträge*, xi, p. 318, 1908.

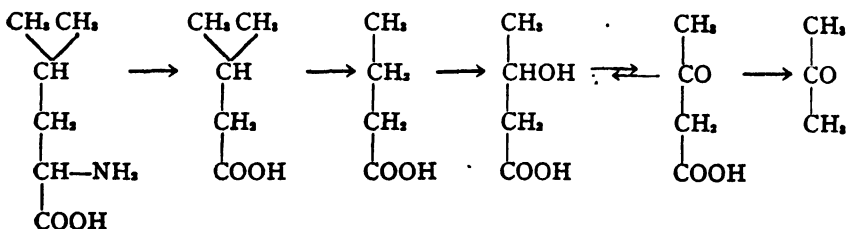
α -amino-butyric nor α -amino-caproic acids showed any evidence of yielding aceto-acetic acid. They drew the conclusion that α -amino-acids suffer oxidation in the α -position giving rise to acids with one carbon atom less, while the carboxyl radical is split off, in all probability in the form of CO_2 .

In the protein molecule we find two amino-acids which contain the isopropyl radical—leucine and valine. Halsey⁹ fed leucine to phlorrhizinized dogs, and, of six feedings, two showed considerable amounts of extra glucose, and four gave entirely negative results. Recently Dakin published one experiment in which he fed 15 grams of *i*-leucine and obtained 4 grams of extra glucose. Neither Halsey nor Dakin are inclined to attribute glucogenetic properties to leucine.

When we consider leucine in the light of our experiments with unsubstituted fatty acids with branched chains, and having in mind Embden's results, in which he showed that leucine is a strong aceto-acetic acid builder, there remains only one possible conclusion with regard to the catabolism of leucine. As far as our knowledge goes, and as will be shown in a following communication, we are fully justified in assuming that all carbons in the α -position having an amino radical lose the amino radical and become oxidized possibly to the carboxyl state, giving rise to compounds (acids) with one carbon less.

For the present we do not include glycocoll or alanine in this category. Experiments are in progress which will throw light on the rôle of the carboxyl in the formation of glucose from these substances.

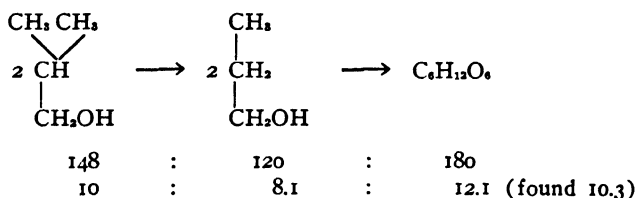
Leucine in its catabolism can be assumed to pass through the following intermediary stages:



⁹ Halsey: *Amer. Journ. of Physiol.*, x, p. 229, 1903.

Ordinarily leucine cannot be classed as a glucose-yielding substance, but under certain circumstances it may give rise to glucose. There are certain flora of intestinal bacteria that seem to possess the power of effecting α -oxidation. In one of our experiments butyric acid, which, when given subcutaneously never yields even a trace of glucose, when fed *per os* gave rise to as much as 3 grams of glucose. This reaction cannot be explained in any other way, and it seems very possible that the same factors obscured Halsey's two experiments.

When we found that isobutyric acid gave rise to glucose and that isobutyl alcohol could yield an amount of glucose that corresponds to the conversion of three of its carbons into glucose, we were led to the belief that valine in its catabolism was broken down to isobutyric acid, and that it would be found to be one of the amino-acids that give rise to glucose.



In his paper on the "Intermediary Metabolism of Amino-acids," Dakin¹⁰ showed that valine yields practically no glucose. This of course makes our theory more difficult of interpretation, but we do not believe it robs the theory of its strength, for the antiketogenic properties of valine are very similar to those of isobutyric acid, and as will be shown in the future, this property is possessed only by compounds capable of forming glucose. (The reverse, however, is not true, *i. e.*, not all compounds that are capable of forming glucose possess antiketogenic properties.)

SUMMARY.

Experiments were performed on phlorhizinized dogs.

I. It was found that isobutyric acid and isobutyl alcohol give rise to glucose, probably by undergoing demethylation and by giving rise to normal fatty acids (propionic acid).

¹⁰ Dakin, *This Journal*, xiv, p. 321, 1913.

II. Isovalerianic acid does not give rise to glucose, but to large quantities of aceto-acetic acid, acetone and β -hydroxybutyric acid.

III. Isocaproic acid was found to give rise to glucose, probably having, by a process of demethylation, formed normal valerianic acid, which became oxidized to propionic acid.

IV. Isobutyric acid, in certain cases, possesses very marked anti-ketogenetic properties.

V. It is suggested that isovalerianic acid is one of the intermediary stages in the catabolism of leucine.

VI. It is also suggested that isobutyric acid may be an intermediary body in the catabolism of valine.

EXPERIMENT XI.
Twelve-Hour Periods.

DATE 1913	PERIOD	WEIGHT	TOTAL NITROGEN	TOTAL GLUCOSE	POLARIZATION	D: N	EXTRA GLUCOSE	NH ₄ N	ACETONE AND ACETOACETIC ACID	β -HYDROXY-BUTYRIC ACID	REMARKS
Feb.											
27	III		3.96	13.76	+0.63°	3.47		0.153	0.128	0.509	1 gm. phlorhizin.
27	IV	8.66	3.45	11.21	+0.50°	3.26		0.226	0.197	1.12	1 gm. phlorhizin.
28	V		3.60	11.50	+0.52°	3.20		0.274	0.223	1.165	10 gms. isobutyric acid given subcutaneously as Na salt in 2 doses.
28	VI	8.32	3.29	14.03	+0.66°	4.26		0.187	0.059	0.238	
March											
1	VII		2.55	10.44	+0.45°	4.11		0.134	0.210	1.325	1 gm. phlorhizin.
1	VIII	8.26	3.36	11.05	+0.46°	3.29		0.266			
2	IX		3.61	11.47	+0.44°	3.18		0.334	0.549	2.75	10 gms. isovalerianic acid.
2	X	8.08	3.24	10.75	+0.41°	3.32		0.218	0.37	2.50	1 gm. phlorhizin.
3	XI		3.31	10.52	3.18	3.18		0.266	0.183	1.14	10 gms. isobutyric acid dissolved in 30 cc. of water; given subcutaneously as Na salt in 2 doses.
3	XII		3.18	12.42	+0.54°	3.91	2.55	0.100	0.059	0.237	
4	XIII		2.60	7.88		3.03		0.157	0.076	0.671	1 gm. phlorhizin.
4	XIV	7.82	2.30	7.62		3.31		0.142	0.219	1.29	
5	XV		2.68	8.35		3.11		0.204	0.262	1.57	1 gm. phlorhizin.

EXPERIMENT XII.
Twelve-Hour Periods.

DATE 1913	PERIOD	WEIGHT	TOTAL NITROGEN	TOTAL GLUCOSE	POLARIZA- TION	D: N	EXTRA GLUCOSE	NH ₄ N	ACETONE AND ACETO- ACETIC ACID	β -HYDROXY- BUTYRIC ACID	REMARKS
Feb.	III										
27	IV	12.70	5.14	18.93	+0.85°	3.69		0.555	0.211	0.830	1 gm. phlorhizin.
27	V	12.70	4.60	16.90	+0.76°	3.68		0.434	0.287	1.598	1 gm. phlorhizin.
28	VI	12.30	5.02	17.54	+0.765°	3.49		0.498	0.328	2.44	10 gms. isovalerianic acid.
28	VII	12.30	3.98	15.55	+0.53°	3.91		0.541	0.037	5.81	1 gm. phlorhizin.
March	VIII	12.30	4.83	17.86	+0.75°	3.70		0.714	0.574	3.58	1 gm. phlorhizin.
1	IX	12.30	4.30	16.37	+0.72°	3.81		0.505	0.410	1.95	1 gm. phlorhizin. 10 gms. isobutyric acid.
2	X	12.38	3.28	17.50	+0.73°	5.34	7.60	0.341	0.281	1.89	subcutaneously.
2	XI	12.08	3.09	14.85	+0.68°	4.82		0.230	0.395	1.29	1 gm. phlorhizin.
3	XII	12.08	3.74	14.84	+0.625°	3.96		0.470	0.606	2.16	10 gms. isobutyric acid given subcutane-
3	XIII	12.08	2.93	13.86		4.75	4.31	0.180	0.642	1.95	ously as Na salt in 2 doses.
4	XIV	11.66	2.96	12.20		4.13		0.174	0.464	1.50	1 gm. phlorhizin.
4	XV	11.66	3.44	11.79		3.43		0.214	0.370	1.445	1 gm. phlorhizin.
5			3.34	10.49		3.14		0.332	0.454	1.74	1 gm. phlorhizin.

EXPERIMENT XIII.
Twelve-Hour Periods.

DATE 1913	PERIOD	WEIGHT	TOTAL NITROGEN	TOTAL GLUCOSE	POLARIZATION	D : N	EXTRA GLUCOSE	NH ₃ N	ACETONE AND ACETO-BUTYRIC ACID	REMARKS
March										
12	II		7.28	24.60	+1.158°	3.38		0.587	0.311	1 gm. phlorhizin.
13	III	15.30	6.90	25.19	+1.20°	3.65	1.95	0.308	0.259	11.6 gms. isocaproic acid given subcutaneously as Na salt.
13	IV		7.14	23.95	+1.15°	3.36		0.383	0.233	1 gm. phlorhizin.
14	V	15.00	6.98	22.26	+0.96°	3.19		0.595	0.297	
14	VI		6.87	27.90	+1.30°	4.06	9.15	0.278	0.299	20 gms. isobutyric acid given subcutaneously as Na salt in 2 doses.
15	VII		7.27	25.65	+1.21°	3.53		0.210	0.320	
15	VIII	14.52	7.61	23.48	+1.02°	3.09		0.415	0.350	1 gm. phlorhizin.
16	IX		6.87	21.18	+1.07°	3.07		0.325	0.347	
16	X		5.98	23.09	+0.90°	3.86		0.292	0.286	1 gm. phlorhizin.
17	XI	13.72	5.25	20.14	+0.86°	3.84		0.265	0.149	
17	XII		4.50	21.38	+0.86°	4.75	10.23	0.293	0.135	10 gms. isobutyl alcohol given subcutaneously.
18	XIII	13.12	4.09	20.40	+0.905°	5.00		0.366	0.185	
18	XIV		5.74	20.09	+0.86°	3.50		0.364	0.178	1 gm. phlorhizin.

EXPERIMENT XIV.
Twelve-Hour Periods.

DATE 1913	PERIOD	WEIGHT	TOTAL NITROGEN	TOTAL GLUCOSE	POLARIZATION	D: N	EXTRA GLUCOSE	NH ₃ N	ACETONE AND ACETO-ACID	β -HYDROXY-BUTYRIC ACID	REMARKS
March											
16	X		4.93	19.45	+0.83°	3.95		0.253	0.219	0.55	1 gm. phlorhizin.
17	XI	11.20	5.42	18.55	+0.77°	3.42		0.359	0.55	2.39	10 gms. isovalerianic acid given subcutaneously as Na salt in 1 dose.
17	XII		5.61	18.59	+0.78°	3.31		0.346	0.455	2.58	1 gm. phlorhizin.
18	XIII	10.68	6.46	21.46	+0.895°	3.35		3.342	0.314	1.66	
18	XIV		5.80	19.70	+0.805°	3.40		0.439	0.330	2.24	1 gm. phlorhizin.
19	XV		5.17	20.67	+0.875°	4.00	3.21	0.252	0.273	1.79	10 gms. isobutyric acid given <i>per os</i> as Na salt.
19	XVI*	{	4.78	16.05	+0.613°	3.36		0.515	0.435	2.87	1 gm. phlorhizin.
20	XVII		4.78	16.05	+0.613°	3.36		0.515	0.435	2.87	
20	XVIII		4.73	14.94	+0.615°	3.16		0.605	0.331	1.93	1 gm. phlorhizin.
21		10.22	3.75	12.24	+0.505°	3.27	2.78	0.270	0.303	1.29	10 gms. isocaproic acid given <i>per os</i> as Na salt.
21	XIX	9.69	3.45	13.29	+0.512°	3.85			0.333		1 gm. phlorhizin.

* Analyzed together.

EXPERIMENT XV.

Twelve-Hour Periods.

DATE 1913	PERIOD	WEIGHT	TOTAL NITROGEN	TOTAL GLUCOSE	POLARIZATION	D. N	EXTRA GLUCOSE	NH ₃ N	ACETONE AND ACETO-ACETIC ACID	β -HYDROXY-BUTYRIC ACID	REMARKS
March	VIII	7.90	2.80	9.44	+0.378°	3.37			0.549	1.935	1 gm. phlorhizin given subcutaneously.
30	IX		2.83	10.51	+0.380°	3.71			0.700	2.080	10 gms. isocaproic acid given per os as Na salt.
31	X					3.84		Some urine lost.			

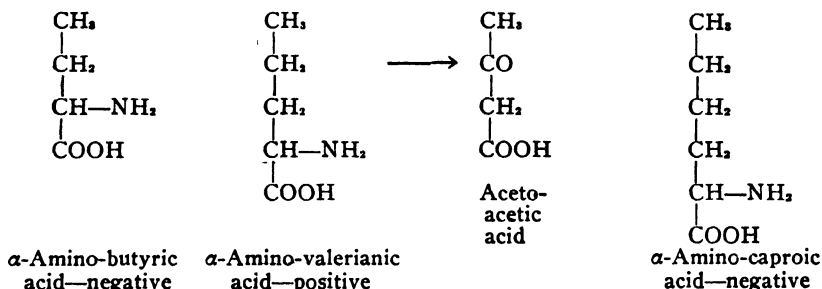
THE CHEMISTRY OF GLUCONEOGENESIS.

IV. THE FATE OF SUCCINIC, MALIC AND MALONIC ACIDS IN THE DIABETIC ORGANISM, WITH CONSIDERATION OF THE INTER- MEDIARY METABOLISM OF ASPARTIC AND GLUTAMIC ACIDS, PROLINE, LYSINE, ARGININE AND ORNITHINE.*¹

By A. I. RINGER, E. M. FRANKEL AND L. JONAS.

(From the Department of Physiological Chemistry of the University of Penn-
sylvania, Philadelphia.)

Embden and Marx² found that on perfusing an extirpated liver with blood to which α -amino-valerianic acid had been added, there was an increase in the aceto-acetic acid formation, while α -amino-butyric acid and α -amino-caproic acid gave negative results.



It is evident that α -amino-valerianic acid must have been changed to a four-carbon compound before it could possibly give rise to aceto-acetic acid. The authors therefore concluded that in α -amino-acids the α -carbon, containing the amino radical, becomes oxidized to a carboxyl state, giving rise to a fatty acid with one carbon less.

Lusk³ found that the feeding of 20 grams of glutamic acid was followed by an elimination of 13.5 grams of extra glucose.

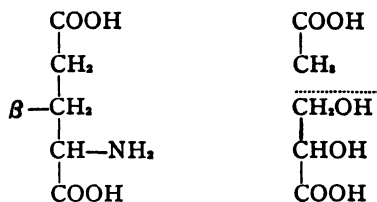
* Received for publication, April 30, 1913.

¹ Aided by a grant from The Rockefeller Institute for Medical Research.

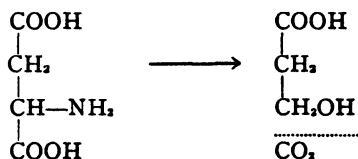
² Embden and Marx: *Hofmeister's Beiträge*, xi, p. 318, 1908.

³ Lusk: *Amer. Journ. of Physiol.*, xxii, p. 174; 1908.

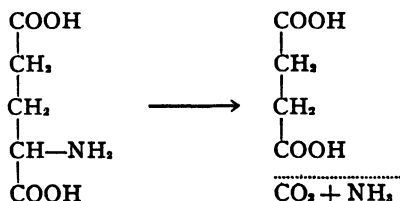
Ringer and Lusk⁴ extended these investigations and found that 20 grams of aspartic acid yielded as much as 14.9 grams of extra glucose. These results corresponded to the conversion into glucose of three of the carbons of either the aspartic or glutamic acid molecule. It was then suggested that glutamic acid undergoes the following changes in the diabetic organism by giving rise to glyceric acid, which was found capable of forming extra glucose.



For aspartic acid it was suggested that the molecule may suffer oxidation in the α -position, *i.e.*, in the carbon containing the amino radical, giving rise to hydrazacrylic acid.



In this series of researches it was our object to test experimentally the paths that these amino-acids may take in their catabolism. In view of the outcome of Embden and Marx' experiments, it suggested itself that glutamic acid may undergo deaminization with oxidation in the α -carbon giving rise to a dibasic acid with one less carbon, *i.e.*, with four carbons, namely, succinic acid. If this were true, succinic acid would yield glucose in the diabetic animal as readily as does glutamic acid.



⁴ Ringer and Lusk: *Zeitschr. f. physiol. Chem.*, lxvi, p. 106, 1910.

In experiment XVI, period IV, 11.8 grams ($\frac{4}{16}$) of succinic acid as sodium salt were given *per os*. The glucose elimination, which was 27.26 in the fore period and 25.02 in the after period, rose to 32.74 grams. The D:N ratio rose from 3.41 to 3.99. The amount of extra glucose eliminated was 5.15 grams. In experiment XVII, period XIII, 11.8 grams of succinic acid as sodium salt were given subcutaneously. The yield of extra glucose was much larger, amounting to 9.45 grams.

These two experiments prove very conclusively that succinic acid can yield large quantities of extra glucose. The question now arises—is it merely an incident that these two substances give rise to glucose or does it actually *prove* that glutamic acid passes through succinic acid in its intermediary stages of metabolism? We believe that the latter is the case, and that glutamic acid *does* give rise to succinic acid for the following additional reason: Succinic acid is found as one of the by-products of alcoholic fermentation. Pasteur⁵ proved its presence conclusively and found that the quantity of succinic acid bore a relationship of 0.4 to 0.7 per cent to the fermented glucose. He believed that succinic acid was a product of the fermentation of the glucose molecule. This theory of Pasteur greatly disturbed the then current conception of Gay-Lussac that a molecule of glucose, in the process of fermentation, breaks down to two molecules of alcohol and two molecules of carbon dioxide.



During the next forty years this was the subject of a great many investigations and slowly evidence accumulated which tended to disprove Pasteur's theory of succinic acid arising from the glucose molecule. It was shown that the quantity of succinic acid was not proportional to the amount of glucose fermented, but to the length of time that fermentation was permitted to go on. Buchner and Meisenheimer⁶ finally succeeded in showing that succinic acid was not a product of glucose fermentation, but a product of the metab-

⁵ Pasteur: *Compt. rend. Acad. Sci.*, 1858 to 1859; *Ann. de chim. et de phys.*, (3) lviii, p. 323, 1860.

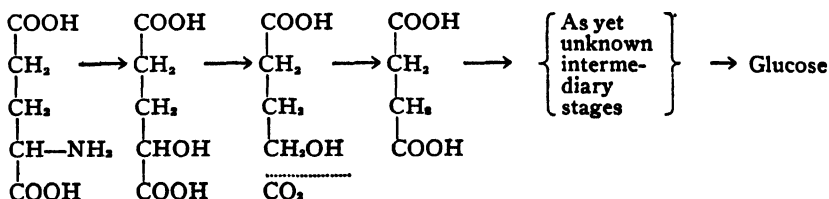
⁶ Buchner and Meisenheimer: *Ber. d. deutsch. chem. Gesellsch.*, xxxix, p. 3201, 1906; xxxiv, p. 1529, 1901.

olism of the yeast cell. Working with expressed cell-free juice of yeast, they could find no succinic acid. The problem of the origin of succinic acid in the by-products of alcoholic fermentation was solved definitely by Felix Ehrlich.⁷ He found that the higher alcohols of fusel oil were the products of the protein metabolism of the yeast cell. He then devoted his researches to finding the mother substances of succinic acid. It was already known at that time that bacteria were capable of effecting deaminization of amino-acids by splitting off the NH_2 radical and converting it into NH_3 substituting a hydrogen for the removed NH_2 .



It was also known that in addition to succinic acid small quantities of aspartic acid were found in the fermentation mixture. It therefore suggested itself to Ehrlich that aspartic acid might be the mother substance of succinic acid and that glutamic acid might be the mother substance of glutaric acid, which would mean, if proven, that yeast cells, like bacteria, are capable of bringing about deaminization in a very simple way—by splitting off NH_2 and substituting a hydrogen for it. When he came to subject these ideas to the test of experimentation, he found that the addition of aspartic acid to fermenting yeast and sugar was followed by no increase in the succinic acid, but when glutamic acid was added, he was able "*stets sehr beträchtliche, den normalen Gehalt weit übersteigende Mengen von Bernsteinsäure, zu isolieren.*"

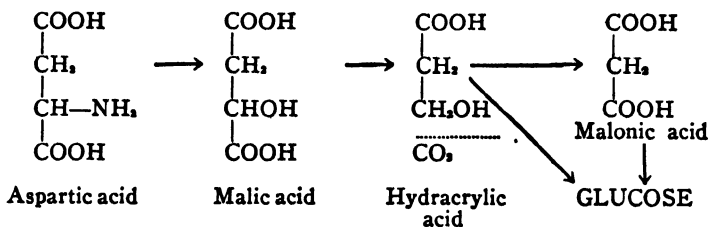
Here we see the direct transformation of glutamic acid into succinic acid by a living cell, and when we compare these results with our own in the diabetic organism, we feel fully justified in concluding that *the path of glutamic acid in metabolism is through succinic acid* undoubtedly passing through α -hydroxy-glutaric acid and γ -hydroxy-butyric acid as intermediary stages:



⁷ Ehrlich: *Biochem. Zeitschr.*, xviii, p. 391, 1909.

THE FATE OF ASPARTIC ACID IN THE DIABETIC ORGANISM.

In the foregoing it was shown that α -amino-glutaric acid (glutamic acid) becomes catabolized to succinic acid, which in turn gives rise to extra glucose. A similar path of catabolism suggests itself for aspartic acid, which is chemically very closely related to glutamic acid.



Aspartic acid, as was shown by Ringer and Lusk,⁸ and asparagine, as was shown by Knopf,⁹ can give rise to large quantities of glucose. In tracing the possible intermediary compounds, one should find all of them capable of yielding glucose to the same extent as does aspartic acid. The first intermediary product of aspartic acid after deaminization appears to be malic acid. In experiment XVI, period II, 13.4 grams ($\frac{M}{10}$) of malic acid as sodium salt were administered subcutaneously. The amount of glucose elimination rose considerably, but not all of it can be attributed to the malic acid, because there was a considerable rise in the protein metabolism of the same period. The D:N ratio rose from 3.4 in the fore period to 3.97 in the experimental period. The amount of extra glucose eliminated was 5.94 grams. Very convincing results were obtained in experiment XX, period VII. 13.4 grams of malic acid, neutralized with calculated amounts of sodium and potassium hydroxide, were given *per os*. The glucose elimination rose from 14 grams in the fore period to 21.14 grams, in spite of the drop of the nitrogen metabolism. The D:N ratio rose from 3.12 to 5.27 and returned in the after periods to 3.27 and 3.22. The calculated extra glucose amounted to 8.32 grams.

⁸ Ringer and Lusk: *loc. cit.*

⁹ Knopf: *Arch. f. exp. Path. u. Pharm.*, xlix, p. 123, 1903.

These two experiments prove very conclusively that malic acid gives rise to glucose to approximately the same extent as does aspartic acid, and that it may be considered a product of aspartic acid metabolism.

The next question to decide was whether hydracrylic acid and malonic acid give rise to glucose. Unfortunately we had none of the former in our possession and were forced to postpone the experiment to a future date. Malonic acid was administered seven times to five different dogs. It was found that because of the large quantities of alkali necessary to neutralize the acid, it was absorbed with great difficulty, and we were never sure that all of the administered material was absorbed. In some cases it was administered *per os*, in others subcutaneously. The oral administrations were always followed by diarrhoea. The amounts of extra glucose produced by malonic acid are here tabulated:

Experiment	XVII	Period	II	Extra	Glucose	0.00	grams.
"	XVIII	"	XVI	"	"	1.00	"
"	XIX	"	IV	"	"	2.64	"
"	XIX	"	VII	"	"	0.65	"
"	XIX	"	X	"	"	2.84	"
"	XX	"	III	"	"	3.06	"
"	XXI	"	III	"	"	1.00	"

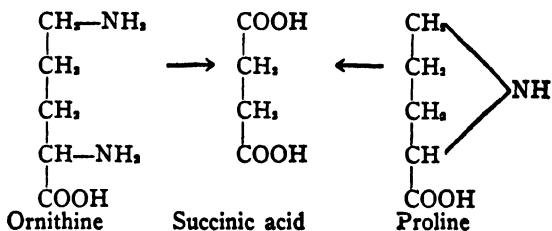
The results are very low indeed, but it is very likely that in the transformation of aspartic acid to a compound with three carbons, the reaction ordinarily does not proceed to a point of complete oxidation of the terminal carbon. It is very likely that hydracrylic acid goes over into glucose before the alcohol radical becomes oxidized to a carboxyl.

INTERMEDIARY METABOLISM OF LYSINE, ARGININE, ORNITHINE AND PROLINE.

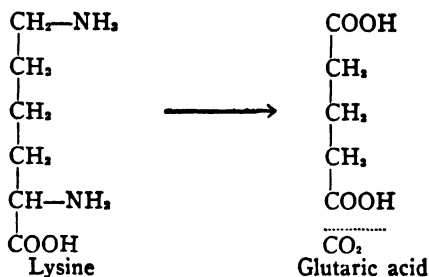
In a recent series of papers, Dakin¹⁰ showed that arginine, ornithine and proline give rise to glucose, when fed to diabetic dogs, while lysine does not give rise to glucose. Dakin rightly suggests that of the arginine only the ornithine moiety goes over into glucose. It

¹⁰ Dakin: this *Journal*, xiii, p. 513, 1913; xiv, p. 321, 1913.

seems to us that ornithine and proline give rise to glucose because of their ability to form succinic acid after undergoing deaminization.



Lysine, on the other hand, after undergoing deaminization, becomes converted into glutaric acid and this was shown not to be convertible into glucose.



SUMMARY.

Experiments were performed on phlorhizinized animals.

I. It was found that succinic, malic and perhaps also malonic acids give rise to extra glucose.

II. Evidence was presented to the effect that succinic acid is an intermediary body in the metabolism of glutamic acid, ornithine and proline, which accounts for the conversion of these substances into glucose.

III. It is suggested that malonic acid may arise in part from the catabolism of aspartic acid.

IV. It was also suggested that lysine in its catabolism passes through a glutaric acid stage, which accounts for its non-conversion into glucose.

EXPERIMENT XVI.

Twelve-Hour Periods.

DATE	WEIGHT	PERIOD	TOTAL NITROGEN	TOTAL GLUCOSE	POLARIZATION	D: N	EXTRA GLUCOSE	NH ₃ N	TOTAL ACETONE	REMARKS
Oct. 1912		I	8.35	28.37	1.096°	3.40		0.56	0.28	13.4 gms. malic acid as sodium salt dissolved in water given subcutaneously. 11.8 gms. succinic acid as sodium salt given <i>per os</i> .
30		II	10.29	40.92	1.635°	3.97	5.94	0.29	0.33	
30	14.49							0.33	0.25	
31		III	7.99	27.26	1.150°	3.41	5.15	0.20	0.37	
31		IV	8.19	32.74	1.402°	3.99				
Nov. 1		V	7.49	25.02	3.34				0.367	

EXPERIMENT XVII.

Twelve-Hour Periods.

DATE	WEIGHT	PERIOD	TOTAL NITROGEN	TOTAL GLUCOSE	POLARIZATION	D: N	EXTRA GLUCOSE	NH ₃ N	TOTAL ACETONE	REMARKS
Nov. 1912		I	3.72	14.38		3.86		0.19	0.56	10.4 gms. malonic acid as sodium salt dissolved in water given subcutaneously.
14	12.90	II	4.20	14.84		3.53	0.0	0.24	0.95	
14								0.22	0.35	
15		III	3.90	14.79		3.79				

EXPERIMENT XVIII.
Twelve-Hour Periods.

DATE	WEIGHT	PERIOD	TOTAL NITROGEN	TOTAL GLUCOSE	POLARIZATION	D : N	EXTRA GLUCOSE	NH ₄ N	ACETONE AND ACETO-ACETIC ACID	β -HYDROXY-BUTYRIC ACID	REMARKS
April 1913											
7		I	6.14	17.85	0.80°	2.92	9.45	0.53	0.47	1.16	11.8 gms. succinic acid as sodium salt dissolved in water, given subcutaneously.
7	12.53	II	5.53	16.84	0.85°	3.05		0.40	0.41	1.14	
8		III	5.35	24.30	1.13°	4.55		0.25	0.37	1.46	
8	12.46	IV	4.47	16.87	0.80°	3.78	1.00	0.28	0.48	1.73	10.4 gms. malonic acid as sodium salt given <i>per os</i> . Diarrhoea.
9		V	4.53	15.43	0.72°	3.41		0.33	0.45	1.85	
9	12.71	VI	4.20	15.95	0.78°	3.80		0.30	0.41	1.56	
10		VII	3.05	11.21	0.62°	3.68		0.22	0.56	2.34	
10	12.32	VIII	3.11	11.76	0.50°	3.78					

EXPERIMENT XIX.
Twelve-Hour Periods.

DATE	PERIOD	WEIGHT	TOTAL NITROGEN	TOTAL GLUCOSE	POLARIZATION	D : N	EXTRA GLUCOSE	NH ₃ N	ACETONE AND ACETO-ACETIC ACID	β -HYDROXY-BUTYRIC ACID	REMARKS
April 1913	I		4.34	14.22	0.625°	3.28		0.27	0.15	0.39	
6	II		5.10	16.55	0.88°	3.24		0.30	0.22	0.54	
7	III		4.99	16.05	0.75°	3.24		0.28	0.25	0.79	
8	IV	6.80	5.21	19.82	0.87°	3.81	2.64	0.23	0.41	1.56	10.4 gms. malonic acid as sodium salt given <i>per os</i> in one dose. One watery movement of bowels.
8	V		5.08	17.36	0.80°	3.42		0.29	0.52	1.72	
9	VI	6.45	4.99	16.86	0.73°	3.38		0.33	0.48	1.87	
9	VII		4.93	17.45	0.78°	3.54	0.65	0.30	0.45	0.98	10.4 gms. malonic acid as sodium salt dissolved in water given subcutaneously.
10	VIII	6.43	4.67	16.05	0.73°	3.44		0.27	0.17	0.51	
10	IX		5.28	16.95	0.75°	3.21		0.29	0.17	0.52	
11	X	6.25	2.88	11.83	0.55°	4.11	2.84	0.13	0.12	0.30	10.4 gms. malonic acid as sodium salt given <i>per os</i> . Diarrhoea.
11	XI		3.79	11.46	0.52°	3.03		0.17	0.21	0.63	
12	XII		3.39	9.60		2.83		0.32	0.10	0.26	

EXPERIMENT XX.
Twelve-Hour Periods.

DATE	PERIOD	WEIGHT	TOTAL NITROGEN	TOTAL GLUCOSE	POLARIZATION	D: N	EXTRA GLUCOSE	NH ₄ N	TOTAL ACETONE	PERFORMED ACETONE	ACETO-ACETIC ACID ACETONE	β-HYDROXY-BUTYRIC ACID	REMARKS
April 1913													
14	I	13.40	7.99	29.35	1.26°	3.67		0.46	0.310	0.061	0.249	1.01	10.4 gms. malonic acid as sodium and potassium salt given subcutaneously in two doses.
15	II		8.13	30.41	1.30°	3.74		0.52	0.365	0.059	0.306	1.28	
15	III	13.09	8.20	33.86	1.50°	4.13	3.06	0.53	0.466	0.061	0.495	1.72	
16	IV		9.08	34.39	1.60°	3.79		0.58	0.457	0.083	0.374	1.74	
16	V	12.91	9.42	36.38	1.62°	3.86		0.64	0.554	0.028	0.526	2.39	
17	VI		7.49	28.25	1.32°	3.78		0.47					

EXPERIMENT XXI.
Twelve-Hour Periods.

DATE	PERIOD	WEIGHT	TOTAL NITROGEN	TOTAL GLUCOSE	POLARIZATION	D: N	EXTRA GLUCOSE	NH ₄ N	TOTAL ACETONE	PERFORMED ACETONE	ACETO-ACETIC ACID ACETONE	β-HYDROXY-BUTYRIC ACID	REMARKS
April 1913													
15	II		4.43	12.50	0.60°	2.82		0.25	0.206	0.129	0.077		10.4 gms. malonic acid as sodium and potassium salt dissolved in water given subcutaneously in two doses.
15	III	10.39	4.93	15.64	0.72°	3.17	1.00	0.32	0.215	0.078	0.137	0.792	
16	IV		4.81	14.90	0.61°	3.10		0.32	0.283	0.087	0.196	1.32	
16	V	10.21	4.32	14.32	0.63°	3.32		0.36	0.271	0.081	0.190	1.86	13.4 gms. malic acid as sodium and potassium salt given <i>per os</i> .
17	VI		4.50	14.00	0.60°	3.12		0.45	0.295	0.066	0.229	1.96	
17	VII	9.94	4.02	21.14	1.04°	5.27	8.32	0.08	0.313	0.125	0.188	1.23	
18	VIII		3.96	12.92	0.40°	3.27		0.32	0.404	0.123	0.371	2.66	
18	IX	9.77	4.20	13.51	0.60°	3.22		0.31	0.500	0.209	0.291	2.28	

ON THE ACTION OF LEUCOCYTES ON HEXOSES.

FOURTH COMMUNICATION. ON THE MECHANISM OF LACTIC ACID FORMATION.*

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New York.*)

It was reported in a previous communication that by the action of leucocytes on *d*-glucose, *d*-mannose or *d*-fructose, *d*-lactic acid was obtained. The mechanism of this transformation may be interpreted by a process consisting of at least three phases: First, transformation of the six carbon chain molecule into two three carbon chains such as glyceric aldehyde; second, into the formation of a substance which would permit a change in the configuration of the groups attached to the α -carbon atom (to the carbonyl group); and third, the transformation of the latter substance into lactic acid. It is evident that the formation of methyl glyoxal in the second phase of the reaction could lead the way to lactic acid formation by enzymes analogous to those which Knoop and Dakin found capable of transforming keto-acids into hydroxy-acids. At the moment when the previous communication was prepared for publication, evidence of the existence of such enzymes was lacking, hence the question was referred to as one requiring further experimental investigation. In fact, experiments in that direction were initiated immediately after the observations reported in the previous communication had been completed.

Simultaneously with our observation on the transformation of the three hexoses into *d*-lactic acid, Dakin and Dudley¹ made the very important discovery of the presence in animal tissues of an

* Received for publication, April 30, 1913.

¹ Dakin and Dudley: this *Journal*, xiv, p. 155, 1913.

enzyme capable of transforming phenyl glyoxal into the natural mandelic acid and later they supplemented it by an observation on the transformation of methyl glyoxal into lactic acid.

The observations of Dakin and Dudley were soon corroborated by Neuberg,² who observed the transformation of pyruvic aldehyde into lactic acid. It seemed at first sight that these observations entirely established the correctness of the view of the mechanism of the transformation of hexoses into lactic acid expressed in our previous communication. However, the conclusion was not absolutely compelling for the following reasons.

First, under the conditions of the experiments of Dakin and Dudley and of Neuberg, hexoses are not converted into lactic acid, but remain intact, and it does not naturally follow that an enzyme present in autolyzing tissues also is present in the uninjured cells. Second, neither Dakin and Dudley nor Neuberg make positive statements regarding the absolute absence of bacterial growth in their solutions. The presence or absence of bacterial contamination is an all important factor for the consideration of the extent to which the observations of Dakin and Dudley and of Neuberg can be applied to the explanation of the mechanism of conversion of the *d*-hexoses into *d*-lactic acid. The enzymes active in the conversion of hexoses into lactic acid bring about the formation of only the dextro-rotatory substance. In the experiments of Neuberg a mixture of *dl* and of the *d* forms was obtained. It is not excluded, under such conditions, that by the action of the enzyme only the racemic acid was formed and that the dextro-rotatory isomer owed its origin to bacterial action. At the time of Dakin and Dudley's first communication the authors brought no evidence of the formation of the optically active acid. Third, in the work of Neuberg there is a lack of evidence that the *d*-lactic acid was not derived from the liver tissue employed in his experiment.

By all this we do not for a moment intend to detract from the importance of the discovery of Dakin and Dudley and of the observations of Neuberg. However, we felt that in order to establish our first view on the mechanism of lactic acid formation from hexoses beyond dispute, it was imperative to demonstrate the conver-

² Neuberg: *Biochem. Zeitschr.*, xlix, p. 502.

sion of methyl glyoxal into *d*-lactic acid under the same conditions which enabled us to bring about the conversion of *d*-hexoses into *d*-lactic acid. For these reasons, and with the consent of Dr. Dakin, we continued our experiments which were in progress at the time of his first publication.

Our experiments were performed under exactly the same conditions as those for the conversion of hexoses into lactic acid. There was an absolute absence of bacterial growth, both aerobic and anaerobic. Leucocytes and kidney tissue were employed in the experiments. The conversion of methyl glyoxal into lactic acid took place with either of the tissues. The lactic acid was a mixture of *dl* and of *d* forms. This furnishes convincing evidence to the view that the formation of *d*-lactic acid from the various *d*-hexoses is conditioned by the intermediate formation of methyl glyoxal.



Controls with leucocytes alone and with kidney tissue alone (in quantities equal to those employed in the principal experiments) showed the absence of lactic acid.

EXPERIMENTAL.

Leucocytes.—These were obtained from dogs by injecting turpentine into the pleural cavity, according to the method previously described.

Tissues.—Rabbits were killed by exsanguination and the kidneys removed aseptically, and minced as fine as possible under sterile conditions.

Methyl Glyoxal.—This was prepared from methyl glyoxal-diaceetol according to Meisenheimer.³ The latter compound was obtained by the method of A. Wohl and M. Lange.⁴

Solutions.—The methyl glyoxal (about 1 gram) was dissolved in 10 cc. of distilled water and sterilized by passing it through a Berkefeld filter. The washed leucocytes were suspended in a 1 per cent Henderson phosphate mixture to which had been added cal-

³ Meisenheimer: *Ber. d. deutsch. chem. Gesellsch.*, xlv, p. 2635, 1912.

⁴ A. Wohl and M. Lange: *Ber. d. deutsch. chem. Gesellsch.*, xli, p. 3612, 1908.

cium phosphate, 3 grams to each 100 cc. of solution. The sterile methyl glyoxal solution was added last. The same procedure was adopted in the experiment with the kidneys.

Lactic Acid.—The mixture of methyl glyoxal with leucocytes and kidneys was set aside at 37° for eighteen hours, and then coagulated by heating and with the addition of sufficient phosphoric acid to dissolve the calcium phosphate. The filtered solution was extracted with ether in a von der Heide extracting apparatus after the addition of sodium sulphate and 5 cc. of phosphoric acid. The zinc salt was prepared according to the method previously described.

Bacteriological Examination.—Both aerobic and anaerobic cultures were made of the solutions prior to their coagulation by Dr. J. Bronfenbrenner, and we desire to express our appreciation for his assistance. No bacterial growth was obtained in either instance after forty-eight hours.

Experiment 1. Methyl Glyoxal and Leucocytes.—About 25 cc. of washed leucocytes were suspended in 100 cc. Henderson phosphate solution with 3 grams of calcium phosphate and about 1 gram methyl glyoxal, and allowed to stand for eighteen hours at 37° . There was obtained 0.283 gram zinc lactate.

0.0811 gram of the recrystallized anhydrous salt after ignition gave 0.0275 gram ZnO = 33.80 per cent ZnO . Calculated = 33.40 per cent.

0.2540 gram zinc lactate in 5 cc. water, total weight 5.3622 grams, gave a rotation in a 2 dm. tube of $\alpha = -0.14^{\circ}$ (pure D-light).

Experiment 2. Methyl Glyoxal and Kidneys.—Both kidneys of a rabbit were minced and added to 100 cc. Henderson phosphate solution containing 3 grams of calcium phosphate, and about 1 gram of methyl glyoxal in 10 cc. water added. There was obtained 0.2440 gram zinc lactate.

0.0598 gram of the anhydrous salt after ignition gave 0.0198 gram ZnO = 33.28 per cent ZnO . Calculated = 33.40 per cent.

0.2440 gram zinc lactate dissolved in 5 cc. water, total weight 5.8350 grams, gave a rotation in a 2 dm. tube of $\alpha = 0.10^{\circ}$ (pure D-light.)

ON THE ACTION OF TISSUES ON HEXOSES.*

BY P. A. LEVENE AND G. M. MEYER.

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The process of intermediate carbohydrate metabolism has been analyzed by three different methods. The first consisted in feeding the normal or diabetic individual or animal with carbohydrates and their derivatives, or substances closely related to them. The second method consisted in perfusion of normal surviving organs with the same substances, and the third was based on the action on carbohydrates of tissue extracts, or of tissues in a state of autolysis, in the presence of antiseptics. Each of the three methods has its advantages and disadvantages. The disadvantages of the first are obvious, as it does not permit of getting hold of all the intermediate products and rarely permits of a quantitative analysis of the products. The shortcomings of the perfusion method are in a lesser degree the same as of the previous, and in addition, as was pointed out recently by Embden and his co-workers,¹ even in experiments lasting only two or three hours the danger of bacterial growth in the circulating blood is considerable.

Finally, the third method seemed to be free of the faults of the first two, and seemed to offer the best conditions for quantitative analysis of all products formed in the reaction. For this reason, and for the reason of the comparative simplicity of the experiment, a great number of investigations have been carried out by this method. However, the greatest part of the work on glycolysis by tissues and tissue extracts suffered from either imperfect technique or from lack of critical analysis of the results of experiments. In reality, it was demonstrated that under the usual conditions, namely, in the presence of antiseptics, no actual destruction of the

* Received for publication, May 2, 1913.

¹ *Biochem. Zeitschr.*, xlv, p. 1 et seq., 1912.

carbohydrate molecule takes place. Whenever there was noted in such experiments a decline in the reducing power of the sugar solution this was caused by condensation of the monosaccharide into a polysaccharide, perhaps a disaccharide.

Up to the present date a successful dissociation of a hexose molecule outside of the body was attained only under one definite condition, namely, by the use of leucocytes in a 1 per cent Henderson phosphate solution and in absence of antiseptics. In this manner it was possible to convert hexoses into *d*-lactic acid.

This observation seemed of considerable significance since many French writers, particularly Lépine² and Mayer,³ were inclined to attribute to leucocytes the function of sugar decomposition in the living organism. The experiments presented here were undertaken with the object of testing whether or not the power of converting hexoses into lactic acid was limited to the leucocytes. It was planned to subject the sugars to the action of tissues in a 1 per cent Henderson phosphate solution under perfectly aseptic conditions. It proved to be a comparatively easy matter to secure kidney tissue in a perfectly aseptic condition, but the attempts to perform similar experiments with other organs offered great difficulties, hence it was concluded for the moment to limit the experiments to kidney tissue.

The results of these experiments were identical with those in the leucocytes experiments. Through the action of the kidneys, *d*-glucose, *d*-mannose and *d*-fructose were converted into *d*-lactic acid. There was perhaps a slight difference in the quantity of sugar attacked in the two sets of experiments, the more powerful action being noted in the leucocytes experiments. The lactic acid, as in previously reported experiments, was identified as the zinc salt.

Controls with kidney tissue without the addition of the hexoses showed complete absence of lactic acid.

The fact that also by the action of kidney tissue the three hexoses yield the same *d*-lactic acid carries sufficient evidence to the effect that the mechanism of lactic acid formation is identical in all tissues.

² *Le diabète sucré*, Paris, 1909.

³ *Arch. internat. de physiol.*, ii, p. 131, 1904.

For the bacteriological examination we are indebted to Dr. J. Bronfenbrenner.

EXPERIMENTAL.

Tissues.—Rabbits were killed by exsanguination and the kidneys removed aseptically. These were reduced to small pieces and transferred to the flasks containing the sugar solution.

Solutions.—The sugars were dissolved in the least quantity of water and sterilized. The 1 per cent Henderson phosphate solution was sterilized separately and then mixed with the sterile sugar solutions.

I. Experiments Showing Disappearance of Sugars in Mixtures of Sugar and Kidneys.

d-Glucose.

	SOLUTION USED	NH ₄ CNS	NH ₄ CNS PER CC.	SUGAR	LOSS	PER CENT LOSS
	cc.	cc.		per cent		
a. At beginning of experiment	2	31.90	15.95	5.72		
After thirty-six hours	2	29.60	14.90	5.34	0.38	6.64
After hydrolysis	1	14.80	14.80	5.30		
b. At beginning of experiment	1	16.20	16.20	5.80		
After thirty-six hours	1	15.20	15.20	5.44	0.36	6.21
After hydrolysis	1	15.40	15.40	5.52		
c. At beginning of experiment	2	27.00	13.50	4.84		
After thirty-six hours	2	24.60	12.30	4.41	0.43	8.93

d-Mannose.

d. At beginning of experiment	2	27.60	13.80	4.32		
After thirty-six hours	2	25.00	12.50	3.85	0.27	6.28
e. At beginning of experiment	2	29.00	14.50	4.54		
After thirty-six hours	2	26.20	13.10	4.20	0.34	7.48

d-Fructose.

f. At beginning of experiment	2	31.80	15.90	5.47		
After thirty-six hours	2	29.60	14.80	5.05	0.42	7.68

Methods of Analysis.—Sugar was estimated by reduction of Fehling's solution; the reduced copper was determined by the Volhard method.

Lactic Acid.—The solutions freed from protein were extracted with ether in a von der Heide extractor. The details of the process have been previously described. The lactic acid was determined as the zinc salt.

Bacteriological Controls.—Aerobic and anaerobic cultures were made of all mixtures prior to analysis by Dr. J. Bronfenbrenner and only those which proved free from all contamination were used.

Two kidneys from a rabbit removed aseptically and immediately taken up in 1 per cent Henderson phosphate solution, were allowed to stand at 37° for thirty-six hours, under exactly the same conditions as those to which sugar had been added. There was no measurable reduction of the Fehling's solution either before or after incubation.

II. Experiments Showing the Formation of d-Lactic Acid.

Glucose.

200 cc. of the glucose mixture (c, Experiment I) were extracted with ether and the lactic acid converted into the zinc salt. Yield, 0.4350 gram zinc lactate.

0.1416 gram of the recrystallized and air dried salt lost 0.0180 gram H₂O

on drying at 110° = 12.70 per cent H₂O.

Calculated = 12.88 per cent H₂O.

0.1416 gram dissolved in 2 cc. water, total weight 2.2712 grams, gave a rotation in a 1 dm. tube of $\alpha = -0.40^\circ$.

$$[\alpha]_D^{20} = -6.4.$$

Mannose.

100 cc. of the mannose mixture (e, Experiment I) gave 0.1860 gram zinc lactate.

0.0964 gram recrystallized and air dried zinc salt lost 0.0126 gram H₂O

on drying at 110° = 13.08 per cent H₂O.

Calculated = 12.88 per cent H₂O.

0.0964 gram dissolved in 2 cc. water, total weight 2.069 grams, gave a rotation in a 1 dm. tube of $\alpha = -0.30^\circ$.

$$[\alpha]_D^{20} = -6.4.$$

Fructose.

100 cc. of the fructose mixture (f, Experiment I) gave 0.2083 gram zinc lactate.

0.1328 gram zinc salt lost 0.0168 gram water on drying at

110° = 12.60 per cent H₂O.

Calculated = 12.88 per cent H₂O.

0.0872 gram dissolved in 1 cc. water, total weight 1.8090 grams, gave a rotation in a 0.5 dm. tube of $\alpha = -0.16^\circ$.

$$[\alpha]_D^{20} = -6.6.$$

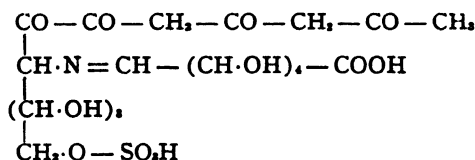
ON CHONDROITIN SULPHURIC ACID.*

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Chondroitin sulphuric acid has been the subject of repeated investigations, yet the information furnished by them added little to that advanced by the work of Schmiedeberg.¹ There is scarcely a new fact brought out by recent investigators which remained unchallenged and undisputed in the light of subsequent investigations.

According to the conception of Schmiedeberg the nucleus of chondroitin sulphuric acid is chondrosin. This, in its acetylated form (chondroitin) combines with sulphuric acid to yield chondroitin sulphuric acid.



The investigation here presented deals primarily with chondrosin. All writers are in accord in the view that chondrosin is composed of two substances in some way or other related to carbohydrates. According to Schmiedeberg the components are glucosamine and glucuronic acid. Orgler and Neuberg² contradicted Schmiedeberg on both points, claiming the components to be aminotetrahydroxycaproic acid and a hexose of undetermined configuration. S. Fränkel³ further modified the view of the two preceding writers in that he interpreted the nature of the nitrogenous body as that

* Received for publication, May 2, 1913.

¹ Schmiedeberg: *Arch. f. exp. Path. u. Pharm.*, xxviii, p. 358, 1891.

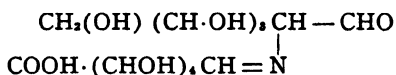
² Orgler and Neuberg: *Zeitschr. f. physiol. Chem.*, xxxvii, p. 407, 1903.

³ S. Fränkel: *Ann. d. Chem.*, cccli, p. 344, 1907.

of an aminoglucuronic acid, the other component supposedly having the structure of a very labile hexose with an undetermined configuration. Still later Kondo,⁴ working in Hofmeister's laboratory, reached the conclusion that one of the components was xylose. Finally reference has to be made to the observation of Mandel and Neuberg⁵ that chondroitin sulphuric acid gave a test with naphtorescorcin characteristic of glucuronic acid.

It must be added here that the only crystalline substances for which absolute purity was claimed by the writer were the salts of tetrahydroxyaminocaproic acid described by Orgler and Neuberg.

It is seen from this brief review that there exists an absolute lack of agreement on the nature of the components of chondrosin. No greater is the harmony of various writers on the mode of union of the two components. According to the view of Schmiedeberg, the carbonyl group of the glucuronic acid is attached to the amino group of the glucosamine, hence giving the following expression to the molecule:



Orgler and Neuberg criticized severely the conception of Schmiedeberg as inconsistent with the properties of the substance, one of the reasons being the observation of Schmiedeberg that the reducing power of a chondrosin solution is not diminished after oxidation with nitric acid; and also on the ground of the great resistance of chondrosin against the hydrolytic action of mineral acid. Orgler and Neuberg, however, do not advance a definite view on the mode of the union of the two components. Also S. Fränkel and Kondo furnish little information in that direction. The latter however argues against the presence of a free carboxyl group in the chondrosin molecule.

In course of the present investigations, which are as yet not completed, definite information was obtained of the nature of one of the components, and also of the condition of some of the characteristic groups of both components.

⁴ Kondo: *Biochem. Zeitschr.*, xxvi, p. 116, 1910.

⁵ Mandel and Neuberg: *Biochem. Zeitschr.*, xiii, p. 148, 1908.

The difficulty of getting hold of the components is conditioned by the fact that chondrosin displays a great resistance towards usual hydrolytic agents, so that it was not possible to bring about hydrolysis of the molecule without simultaneously affecting the integrity of the components. This peculiarity of chondrosin was known to Schmiedeberg.

In course of the present investigation a method was found which brought about a cleavage of the chondrosin molecule permitting the isolation of at least one component. The method consisted in the use of sodium amalgam. The details of the method are described in the experimental part. The substance isolated was the usual glucuronic acid.

The substance was identified by the phenyl and parabromophenylhydrazine derivatives, by the fact that it yielded saccharic acid on oxidation with bromine, as well as by oxidation with nitric acid. The phenylhydrazine derivative had all the properties and the composition of the phenylhydrazid of the osazone. The substance was found to be identical with the one obtained under similar conditions from glucuron and first described by Thierfelder.

The *p*-bromophenylhydrazine derivative had the composition of the substance described by Guido Goldschmiedt and Ernest Zerner.⁶ Under the same conditions we were able to obtain the identical body from pure glucuron. In accord with Goldschmiedt and Zerner we were unable to obtain the substance described by Neuberg. However, the formation of a derivative with only one molecule of hydrazine to one molecule of glucuron is *a priori* not impossible. We made no special effort in obtaining it for the reason that Goldschmiedt and Zerner's substance formed very readily from both pure glucuron and from the hydrolytic products of chondrosin.

Regarding the mode of the union between the glucuronic acid and the second component it was established first that the glucuronic acid is not bound to the amino group of the second component for the reason that the presence of an unsubstituted amino group in the chondrosin was demonstrated by the nitrous acid process.

Further, it was also made obvious that the carbonyl group of

⁶ Goldschmiedt and Zerner: *Ber. d. deutsch. chem. Gesellsch.*, xlii, p. 113, 1913.

the glucuronic acid does not take part in the linking of the two components. The reasons are the following. On oxidation of chondrosin with nitric acid a product is obtained which on distillation with hydrochloric acid gives rise to a minimal quantity of furfural, while the original substance yields a quantity required by a complex composed of one molecule of glucuronic acid and one of a carbohydrate of approximately equal molecular weight. The same oxidation product does not contain free saccharic acid, which could be identified as the acid potassium salt. However, the salt is readily obtained as soon as the oxidation product is hydrolyzed with alkali. Hence, the original oxidation product contains saccharic acid in conjugated form. This in its turn carries convincing evidence that the carbonyl group of the glucuronic acid is not the place of the union between the two components.

Whether or not the carboxyl group of the glucuronic acid is free or serves for linking the two components cannot be stated with certainty at present. Decision has to be postponed until the chemical nature of the second constituent is established. Chondrosin apparently contains only one free carboxyl group, and if the second component contains none then the conclusion will be obvious, that the carboxyl group of the glucuronic acid is present in the chondrosin molecule in a free state.

The fact that chondrosin contains both the carboxyl and the amino groups in a free state, while chondroitin sulphuric acid does not possess reducing properties, and does not react with nitrous acid to form nitrogen gas, indicates that both groups are combined in the more complex molecule with other radicals.

EXPERIMENTAL.

Preparation of Chondroitin Sulphuric Acid Barium Salt.

Nasal septums of cattle were freed from bone and other extraneous material and ground through a meat chopper. Portions of 5 kgm. each were allowed to stand for two days with 10 liters of 2 per cent potassium hydrate solution. The extract was strained through a cloth and the residue again subjected to the same treatment with 5 liters of potassium hydrate solution and finally washed

once with water. The united extracts were acidified with acetic acid and concentrated on the steam bath with an excess of barium carbonate to about half of their volume. The clear liquid was then poured off and the residue thrown on a folded filter and allowed to drain off. This filtrate was united with the decanted liquid and the whole acidified with acetic acid and evaporated as before with barium carbonate to about 2 liters.

The separated protein and barium carbonate were removed by centrifugalization and the clear yellow liquid dropped into eight times its volume of glacial acetic acid and kept agitated by a turbine. The acid potassium salt thus obtained was filtered by suction, washed with glacial acetic acid and finally with alcohol and ether.

Two hundred grams of this product, which gave a slight biuret test, were dissolved in 10 liters of water and while the solution was kept stirred with a turbine, a solution of basic lead acetate was dropped in until complete precipitation had taken place. The lead salt, after having been washed several times by grinding in a mortar with water and filtering with suction, was suspended in 5 liters of water and with the addition of 100 grams of barium acetate and 50 cc. of acetic acid decomposed by long treatment with hydrogen sulphide with constant stirring. After standing for twelve hours the lead sulphide was filtered off and the slightly turbid solution of the barium salt precipitated by the addition of about one-third of its volume of 95 per cent alcohol. After filtering and washing with 50 per cent alcohol, then with 95 per cent, and finally with absolute alcohol and ether, the product after drying was a pure white powder, showing no trace of biuret.

This product is a mixture of the barium salts of chondroitin and chondroitin sulphuric acid. It showed no reduction of Fehling's solution and in the apparatus of Van Slyke no amino nitrogen.

0.5070 gram substance gave 7.75 cc. $\frac{N}{10}$ NH_3 .

0.4650 gram substance gave 7.40 cc. $\frac{N}{10}$ NH_3 .

0.4166 gram substance gave 0.1125 gram BaSO_4 .

	Calculated for $\text{C}_{11}\text{H}_{17}\text{NSO}_{11}$.	Found.
N	2.01 per cent.	(1) 2.14 per cent.
		(2) 2.23 per cent.
S	4.60 per cent.	3.72 per cent.

Preparation of Chondrosin.

Fifty grams of the barium salt of chondroitin sulphuric acid were dissolved in 150 cc. of equal parts of concentrated hydrochloric acid and water, and heated for an hour on the water bath. Barium sulphate begins to separate at once, and after one hour the solution, which is only slightly colored, shows its maximum reduction of Fehling's solution, and all the nitrogen is present as amino nitrogen. The filtered solution was evaporated in vacuum to a very thick syrup and this was taken up in about 40 cc. of hot water and poured into 500 cc. of absolute alcohol. Partial precipitation of the chondrosin hydrochloric acid salt as a nearly colorless flocculent precipitate takes place. After standing over night, two volumes of absolute ether were added and the precipitate filtered with suction and thoroughly washed with absolute ether. For a final purification the product thus obtained is dissolved in about its own weight of water and precipitated and washed again as above described. It is a quite colorless powder, which when properly washed is not hygroscopic. The yield of the first product, dried over calcium chloride for two days in vacuum, was 27 grams.

0.1966 gram substance dried to constant weight at 100° gave 12.7 cc. amino N at 21°, 764 mm. $N = 3.67$ per cent.

0.3319 gram chondroitin sulphuric acid barium salt gave 5.8 cc. $\frac{N}{10}$ NH_4 . $N = 2.44$ per cent.

0.5044 gram substance hydrolyzed for one hour with one part HCl and one part H_2O gave 18.3 cc. amino N at 16°, 760 mm. $N = 2.11$ per cent.

Cleavage of Chondrosin with Sodium Amalgam.

Twelve grams of chondrosin hydrochloride in 100 cc. of water were allowed to stand with 100 grams of 2.5 per cent sodium amalgam. After about twenty minutes at ordinary temperature the solution takes on a bright yellow color and at the same time an evolution of ammonia begins. The solution is then neutralized with sulphuric acid and 100 grams of sodium amalgam are again added, the temperature always being kept at about 25°. After about one hour the solution is again acidified with sulphuric acid and allowed to stand over night, after the addition of a third 100 grams of amalgam. The solution is then separated from the mer-

cury and filtered from the sodium sulphate with the addition of some animal charcoal.

Preparation of Phenylhydrazine Compound.

The solution obtained by the above treatment was diluted to about 200 cc. and after the addition of 15 grams of phenylhydrazine in 50 per cent acetic acid allowed to stand on the water bath. After about 20–30 minutes a dark tarry material has separated together with a small amount of solid material. At this point the solution is quickly filtered with suction on a hot funnel into a hot flask and the filtrate allowed to stand for two to three hours on the water bath. After this time the solution is filled with long yellow needles to which very little of the light-colored oil adheres. The crystals were filtered and washed with warm water and then with cold absolute alcohol until no more oil drops could be discerned under the microscope. When dried in vacuum the product melts with decomposition at about 122°. Attempts to recrystallize did not effect a purification and therefore the first product was used for the analysis.

0.1188 gram substance gave 0.2484 gram CO₂; 0.0634 gram H₂O.

0.1278 gram substance gave 19.2 cc. N, 17°, 758 mm.

	Calculated for C ₂₄ H ₂₈ N ₄ O ₄ · 5H ₂ O.	Found.
C	58.93 per cent.	58.80 per cent.
H	5.93 per cent.	6.12 per cent.
N	17.17 per cent.	17.33 per cent.

0.0599 gram substance in 5 cc. pyridine alcohol mixture rotated in a 0.5 dm. tube with D-light — 0.32°.

Phenylhydrazine Compound from Glucuron.¹

One gram glucuron was warmed on the water bath for two hours with a little more than the required amount of normal sodium hydrate. The solution was neutralized with acetic acid, and 4 grams of phenylhydrazine in 50 per cent acetic acid and 4 grams of sodium acetate were added. After a short time crystallization of the phenylhydrazine compound in long yellow needles began and after three hours their amount had reached 1.6 grams. The material

¹ Thierfelder: *Zeitschr. f. physiol. Chem.*, xi, p. 395, 1887.

was purified by washing with cold alcohol and ether. It decomposed at about 122° .

0.0598 gram substance in 5 cc. pyridine alcohol mixture rotated in a 0.5 dm. tube with D-light— 0.322° .

By prolonged heating in vacuum at 100° the substance loses weight but before becoming constant decomposition sets in, while at lower temperatures no loss of weight was observed.

Parabromphenylhydrazine Compound from Chondrosin.

Twenty grams of chondrosin hydrochloride were treated in the usual way with sodium amalgam, and the resulting solution, after acidifying with acetic acid, heated on the water bath with 4 grams of parabromphenylhydrazine hydrochloride. After about one hour the solution was filtered from the separated tarry material and allowed to stand three hours longer on the water bath. The impure phenylhydrazine compound obtained was washed with alcohol until the impurities had been removed and then with ether. The substance may be recrystallized by dissolving in as little as possible of a mixture of one part of 50 per cent acetic acid and one part alcohol and then precipitating by the addition of two parts of hot water.

0.0568 gram of the substance in 5 cc. pyridine alcohol mixture rotated with D-light in a 0.5 dm. tube— 0.8° .

0.0614 gram twice recrystallized under the same conditions rotated— 0.75° .

0.1454 gram substance gave 12.5 cc. N, 22° , 762 mm.

0.1126 gram substance gave 0.0118 gram AgBr.

	Calculated.	Found.
Br	28.95 per cent.	27.00 per cent.
N	10.15 per cent.	9.72 per cent.

Parabromphenylhydrazine Compound from Glucuron.

One gram of glucuron in 100 cc. of water was heated on the water bath with 2.5 grams of parabromphenylhydrazine hydrochloric acid salt, which had been purified by twice recrystallizing from dilute hydrochloric acid and washing with ether, and 2.5 grams of sodium acetate. After about one hour 0.3 gram of a yellow crystalline substance had separated. The mother liquor

filtered from the first crystallization gave upon further heating 0.2 gram more of the same substance. After recrystallization from 50 per cent acetic acid and alcohol it had the following composition.

0.1436 gram substance gave 13 cc. N at 22°, 758 mm.

0.1268 gram substance gave 0.0824 gram AgBr.

0.1338 gram substance gave 0.0124 gram Na_2SO_4 .

	Calculated for $\text{Br}_7\text{C}_{18}\text{H}_{17}\text{O}_8\text{N}_7\text{Na}$.	Calculated for $(\text{C}_{18}\text{H}_{17}\text{N}_7\text{O}_7\text{Br})$.	Found.
Br	28.95 per cent.	20.97	27.65 per cent.
Na	4.17 per cent.		3.01 per cent.
N	10.15 per cent.	7.21	10.20 per cent.

0.0653 gram substance in 5 cc. pyridine alcohol mixture rotated in a 0.5 dm. tube with D-light — 0.90°.

Nitric Acid Oxidation of the Products of Hydrolysis of Chondrosin.

Twenty-five grams of chondrosin hydrochloride were treated with sodium amalgam in exactly the same manner as described in the previous experiment. The solution, after having been freed from inorganic salts by precipitation with alcohol, was evaporated to a syrup. This syrup was quickly evaporated in a flat dish with nitric acid composed of one part of nitric acid, specific gravity of 1.42, and one part of water. The residue was then evaporated several times with water and finally taken up in 15 cc. of water and neutralized with potassium hydrate. Upon addition of glacial acetic acid the crystallization of the acid potassium saccharate began after a short time. After two days the yield amounted to 1.1 grams. For analysis it was recrystallized from water.

0.1253 gram substance gave 0.0427 gram K_2SO_4 .

	Calculated.	Found.
K	15.72 per cent.	15.32 per cent.

Nitric Acid Oxidation of Chondrosin and Subsequent Hydrolysis.

Ten grams of chondrosin hydrochloride were evaporated in a flat dish on a water bath with 10 cc. of nitric acid and 10 cc. of water. The residue was dissolved in 10 cc. of water and 5 cc. of nitric acid and again evaporated to dryness. The final residue

was then dissolved in 10 cc. of water and the solution divided into two parts of 7 and 3 cc. each and neutralized in the cold with potassium hydrate. The larger portion, after addition of 2 cc. of 50 per cent potassium hydrate, was allowed to stand for two hours on the water bath and then acidified with acetic acid. After several hours the acid potassium saccharate began to separate. The yield amounted to 0.5 gram after two days. From the smaller portion, after addition of acetic acid, only a trace of the same substance separated after long standing.

0.1276 gram substance gave 0.0440 gram K_2SO_4 .

	Calculated.	Found.
K	15.72 per cent.	15.46 per cent.

Brom Oxidation of the Products of Hydrolysis of Chondrosin.

A solution of 25 grams of chondrosin hydrochloride was treated in the usual way with 2.5 per cent sodium amalgam. The solution was acidified with hydrochloric acid and allowed to stand for five days at ordinary temperature with an excess of bromine. It was then concentrated in vacuum to about 100 cc. and the principal amount of the salt separated by pouring the substance into hot absolute alcohol. The alcoholic solution was concentrated in vacuum to a syrup, taken up in water, and the halogen determined in an aliquot part. The requisite amount of lead acetate was then added to the remainder of the solution and the lead chloride and bromide removed by filtration. The excess of lead was then removed by hydrogen sulphide and the solution evaporated in vacuum to about 30 cc. It was then neutralized with potassium hydrate and after the addition of 10 cc. of glacial acetic acid allowed to stand for two days in the refrigerator. The separated crystals were filtered on suction and the product recrystallized from water. After drying it amounted to 1.6 grams.

0.1209 gram substance gave 0.0419 gram K_2SO_4 .

0.1210 gram substance gave 0.0466 gram H_2O ; 0.1242 gram CO_2 .

	Calculated for $C_8H_{13}O_8K$.	Found.
H	3.65 per cent.	4.28 per cent.
C	27.90 per cent.*	27.98 per cent.
K	15.72 per cent.	15.55 per cent.

* Considering that one atom of carbon is contained in the ash as K_2CO_3 .

Furfurol from Chondrosin after Oxidation with Nitric Acid.

0.4219 gram of chondrosin (calculated from the nitrogen content) was evaporated to dryness with 5 cc. concentrated nitric acid and 5 cc. of water. After repeated evaporation with water the solution of the residue was distilled in the usual way with hydrochloric acid of specific gravity 1.06, until no more furfurol was given off. Upon addition of 0.1 gram of phloroglucin 0.0076 gram of phloroglucoside was obtained, corresponding to 0.0218 gram of glucuronic acid, or about one-tenth of the amount present in chondrosin.

Desamido Chondrosin.

Three grams of chondrosin hydrochloride in 50 cc. of water were treated with the calculated amount of silver nitrite (1.1 grams). After standing for several hours at ordinary temperature the reaction mixture was warmed on the water bath with occasional shaking. After the solution had been allowed to stand over night at ordinary temperature it was again warmed on the water bath for about two hours, after addition of 0.3 gram of silver nitrate and about 5 cc. of diluted hydrochloric acid. The excess of silver was then removed with a slight excess of hydrochloric acid and the solution evaporated in vacuum to a syrup which was taken up in very little water and poured into dry acetone. The gummy precipitate hardened quickly and was then ground with more dry acetone and washed with ether. The product was then a white amorphous powder resembling chondrosin in all its physical properties and its power to reduce Fehling's solution and gave the same amount of furfurol.

0.3710 gram substance dried at 100° in vacuum gave 0.0575 gram phloroglucoside corresponding to 0.1725 gram glucuronic acid.

THE CHEMISTRY OF GLUCONEOGENESIS.

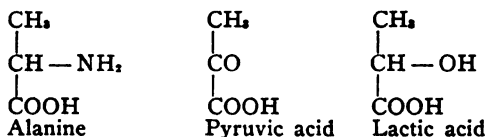
V. THE RÔLE OF PYRUVIC ACID IN THE INTERMEDIARY METABOLISM OF ALANINE.*

BY A. I. RINGER.

WITH THE ASSISTANCE OF E. M. FRANKEL AND L. JONAS.

(From the Department of Physiological Chemistry of the University of Pennsylvania, Philadelphia.¹)

Pyruvic acid occupies a singularly important position in intermediary metabolism. Because of its chemical relationship to alanine and lactic acid



it is assumed to play a rôle in the intermediary metabolism of both protein and carbohydrates.

Not until recent years was any attempt made to study the paths that the amino-acids undergo in the process of their catabolism. All that was known was that the amino-acid broke down into a nitrogenous fraction that gave rise to urea and ammonia, and a "nitrogen-free" fraction which was "burnt."

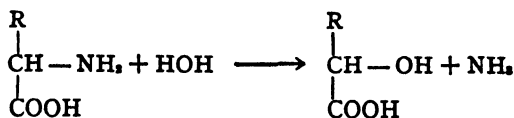
With the development of our knowledge of the structural composition of the amino-acids and their related compounds, evidence began to accumulate which suggested definite reactions and definite paths of decomposition. Until the researches of Neubauer² came to light, it was the current belief that the α -amino-acids suffered deamination in the animal body by a process of hydrol-

* Received for publication, May 27, 1913.

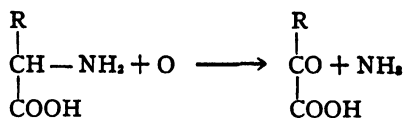
¹ Aided by a grant from The Rockefeller Institute for Medical Research.

² O. Neubauer: *Deutsch. Arch. f. klin. Med.*, xcv, p. 211, 1909.

ysis, whereby the NH_2 was removed and an hydroxyl took its place.



Neubauer was the first to call attention to a different process of deamination, *i. e.*, oxidative deamination,

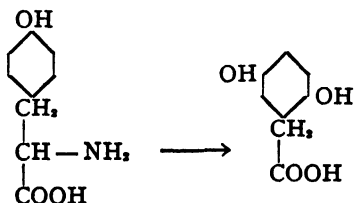


which has so much experimental evidence to support it that it is now almost universally accepted.

We shall not attempt to present a detailed account of the experiments which led to this conclusion, but will briefly state the facts that have a bearing on our present discourse.

Neubauer worked on a patient suffering from alkaptonuria. Such a patient presents an abnormality in his protein metabolism, which consists of his inability to burn tyrosine and phenylalanine and of the excretion of homogentisic acid in the urine. Neubauer and his associates utilized this fact in their study of their intermediary metabolism of tyrosine and phenylalanine³ in the course of which they came to the following conclusions:

I. That tyrosine (para-oxyphenyl- α -amino-propionic acid) gives rise to extra homogentisic acid⁴



³ O. Neubauer and W. Falta: *Zeitschr. f. physiol. Chem.*, xlii, p. 81, 1904.

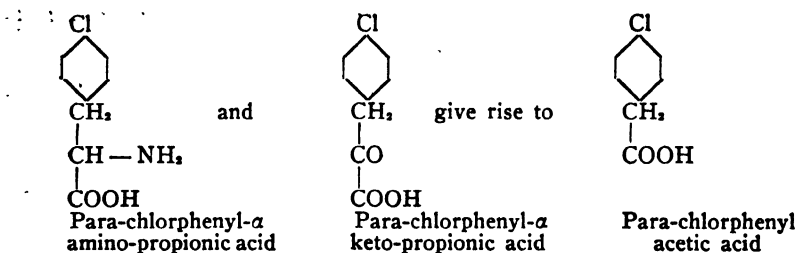
⁴ Wolkow and Baumann: *ibid.*, xvi, p. 270, 1892.

II. That para-oxyphenyl- α -hydroxy-propionic acid, $\text{HO.C}_6\text{H}_4\text{.CH}_2\text{.CHOH.COOH}$, does not give rise to extra homogentisic acid.

III. That para-oxyphenyl- α -keto-propionic acid, $\text{HO.C}_6\text{H}_4\text{CH}_2\text{.CO.COOH}$, gives rise to extra homogentisic acid.

Since these α -amino and α -keto compounds can give rise to homogentisic acid, and the α -hydroxy cannot, Neubauer concluded that the α -amino compound cannot possibly give rise to the α -hydroxy acid as an intermediary body. He then conceived of the "oxidative deamination" theory, which seems to explain his findings beautifully.

Additional support to this theory was rendered by Friedmann and Masse⁵ who showed that



whereas para-chlorophenyl- α -hydroxy-propionic acid, $\text{Cl.C}_6\text{H}_4\text{.CH}_2\text{.CHOH.COOH}$, did not give rise to para-chlorophenyl acetic acid. This again showed that the hydroxy acid could not possibly have been an intermediary compound in the metabolism of the amino acid.

On the strength of his findings Neubauer was led to extend his theory to the entire series of α -amino-acids and suggested that alanine, in the animal body, gives rise to pyruvic acid, which may secondarily give rise to lactic acid.

Reviewing the evidence in support of this theory, one feels convinced of the soundness of the conclusion in the case of the aromatic compounds. With regard to the open chain α -amino-acids, however, there seems to be little direct evidence. It is true that the conversion of α -keto-acids into alanine by the animal organism has

⁵ Friedmann and Masse: *Biochem. Zeitschr.*, xxvii, p. 97, 1910.

been proven and that ketonic acids may in the animal body go over with great ease into the corresponding hydroxy acids, which process has been shown repeatedly to be reversible; still we feel that *it is not proven satisfactorily that alanine in its catabolism must pass through pyruvic acid, and that lactic acid can arise only secondarily from pyruvic acid.*

We raise this question because in experiments which we have performed with the object of ascertaining the degree to which pyruvic acid can give rise to extra glucose in diabetic animals, it was found that pyruvic acid does not behave in the way it would if it really occupied a definite and obligatory place in the intermediary metabolism between alanine and lactic acid.

During the course of our work we have come across several substances whose gluconeogenetic properties are not constant, which have the power of yielding more glucose at one time and less at other times, and we have been inclined to attribute their gluconeogenetic properties to certain factors of equilibrium, which turn the reaction of their metabolism in one path at one time and in another path at another time. According to our experience pyruvic acid may be classed among this group.

The status of pyruvic acid in metabolism has been the subject of very considerable discussion during the past year. Parnas and Baer⁶ perfused the liver of the tortoise with Ringer's solution, blood and oxygen to which 4 grams of pyruvic acid as sodium salt had been added, and found no increase in the glycogen. On the basis of this one experiment they drew the conclusion that pyruvic acid cannot be classed among the glucogenetic substances. In our estimation, this conclusion is not at all justified.

P. Mayer⁷ administered 7 to 8 grams of pyruvic acid to normal rabbits and found that they developed glucosuria, the severity of which depended upon the state of nutrition of the animal. In one rabbit he obtained 2.4 grams of glucose in the twenty-four hours following the administration of pyruvic acid. In animals which had fasted for periods of ten to eleven days, the administration of pyruvic acid was not followed by glucosuria, but by an increase of the glucose concentration of the blood and by an increase in the

⁶ Parnas and Baer: *Biochem. Zeitschr.*, xli, p. 386, 1912.

⁷ Paul Mayer: *ibid.*, xl, p. 441, 1912.

glycogen in the liver. In this communication Mayer left open the question of the origin of the glucose. In a second paper on the subject⁸ (which appeared after our work was far advanced) Mayer studied the influence of pyruvic acid on gluconeogenesis in phlorhizinized dogs and rabbits. He found that pyruvic acid did not give rise to any extra glucose, and that in some cases the urinary constituents were greatly diminished after the pyruvic acid administration. In experiment X the glucose elimination dropped from 13.48 grams in the fore period to 1.45 grams (!) in the experimental period, and the nitrogen dropped from 2.92 grams to 0.53 gram (!). In experiment XI the glucose elimination dropped from 32.93 grams to 3.41 grams (!). The kidneys of these animals were examined microscopically and extensive tubular degeneration was found. Mayer concluded that pyruvic acid is a toxic substance which causes a disturbance in the sugar and nitrogen elimination and which acts by decreasing the permeability of the kidneys.

To all of these conclusions we object most emphatically. We gave Kahlbaum's pyruvic acid seven times to six different dogs in quantities varying from 8.8 to 13.2 grams, administered subcutaneously and orally, and never have we obtained any of the toxic symptoms described by Mayer. In no case did we get the peculiar drop in the glucose and nitrogen eliminations, and in no case did we observe any sign or symptom of any kidney involvement. We have, however, seen a picture of Mayer's experiences with pyruvic acid after subcutaneous and, under certain circumstances also, after oral administration of tartaric acid.⁹ The resemblance is so close that we do not hesitate for a moment to attribute Mayer's results to a contamination of his pyruvic acid with tartaric acid. This is all the more probable since pyruvic acid is very largely prepared by the distillation of tartaric acid.

In all of our experiments pyruvic acid appears to be a glucose-yielding substance, the question is only one of degree. The methods employed in these experiments are the same as those employed and described in the previous papers of this series.

⁸ Paul Mayer: *ibid.*, xlix, p. 486, 1913.

⁹ Underhill: this *Journal*, xii, p. 115, 1912. We have been able to corroborate Underhill's findings. Our results will be published soon.

In experiment XXII period VIII, 10 grams of pyruvic acid as sodium salt were administered subcutaneously. The glucose elimination rose from 33.6 grams in the fore period to 40.91 grams in the experimental period, and returned to 35.75 and 32.54 grams in the after periods IX and X respectively. The amount of extra glucose eliminated was 8.21 grams.

In experiment XXIII period III, 10 grams of pyruvic acid as sodium salt were given subcutaneously. The glucose elimination rose from 28.54 to 32.04 grams and returned to 22.84 grams in the after period. The D:N ratio, which was 3.44 in the fore period, rose to 4.18 and came down to 3.6 in the after period. The amount of extra glucose was 5.09 grams.

In experiment XXIV period III, 13.2 grams of pyruvic acid as sodium salt were given subcutaneously. The glucose elimination rose from 17.58 grams to 19.18 grams. The amount of extra glucose eliminated was 2.25 grams.

In experiment XXV period IV, 8.8 grams of pyruvic acid as sodium salt were given subcutaneously. The amount of extra glucose eliminated was 5.1 grams.

In experiment XXVI period V, 8.8 grams of pyruvic acid as sodium salt were given subcutaneously. The amount of extra glucose eliminated was 1.16 grams.

In two other experiments, 10 grams of pyruvic acid as sodium salt were given *per os*. The dog vomited part of the ingested material each time. The animals lived for a long time afterwards and no change in the urinary constituents was noticed. The experiments are not reported in detail because the vomitus contaminated part of the urine.

From all these experiments we see very clearly that pyruvic acid cannot be considered a toxic substance (in Mayer's sense) and that in most of the cases pyruvic acid yields large quantities of extra glucose.

However, when we come to compare the results obtained after pyruvic acid administration with those obtained after alanine¹⁰ and lactic acid,¹¹ we note a very marked difference. While alanine

¹⁰ Ringer and Lusk: *Zeitschr. f. physiol. Chem.*, lxvi, p. 106, 1910.

¹¹ Mandel and Lusk: *Amer. Journ. of Physiol.*, xvi, p. 129, 1906.

and lactic acid never fail to yield large quantities of extra glucose, pyruvic acid at times yields very small quantities (experiments XXIV and XXVI). This fact makes it very certain that *in the process of catabolism alanine cannot have pyruvic acid as its principal product of intermediary metabolism, and that alanine does not undergo oxidative deaminization.* Alanine and lactic acid yield glucose in quantities so similar to each other, that one seems justified in concluding that the conversion of the former into the latter is quantitative.

In a previous communication¹² it was shown that malic acid yields glucose in quantities similar to aspartic acid, and it was suggested that the former was an intermediary product in the metabolism of the latter. It becomes of interest to know what rôle, if any, the corresponding ketone—oxalacetic acid—plays in it. These experiments are in progress and will be reported soon.

EXPERIMENT XXII.

Twelve-Hour Periods.

DATE 1913	PERIOD	WEIGHT	TOTAL NITROGEN	TOTAL GLUCOSE	D : N	EXTRA GLUCOSE	NH ₃ N	ACETONE AND ACETO- ACETIC ACID	REMARKS
Feb. 2	IV		9.82	38.38	3.91		0.89	1.25	
3	V		9.12	33.60	3.68		0.88	1.04	
3	VI	17.40	8.04						
4	VII		8.00						
4	VIII	17.00	8.65	40.91	4.73	8.21	0.505	0.308	10 gms. pyruvic acid as Na salt given subcutaneously.
5	IX		9.18	35.75	3.89		0.50	0.279	
5	X	16.64	8.62	32.54	3.77		0.49	0.283	

EXPERIMENT XXIII.

Twelve-Hour Periods.

3	II	12.40	8.28	28.54	3.44			0.340	
3	III		7.66	32.04	4.18	5.09		0.160	10 gms. pyruvic acid as above.
4	IV		6.34	22.84	3.60			0.430	
4	V	11.54	6.13	23.38	3.81			0.501	
5	VI		5.81	20.51	3.52			0.840	
5	VII	11.20	5.32	20.08	3.77			0.514	
6	VIII		5.21	18.40	3.53				

¹² Ringer, Frankel and Jonas: this *Journal*, xiv, p. 539, 1913.

EXPERIMENT XXIV.

Twelve-Hour Periods.

DATE 1913	PERIOD	WEIGHT	TOTAL NITROGEN	TOTAL GLUCOSE	D : N	EXTRA GLUCOSE	NH ₄ N	ACETONE AND ACETO- ACETIC ACID	β -OXY BUTYRIC ACID	REMARKS
Apr. 30	I		4.77	18.45	3.87		0.27	0.07	0.24	
May 1	II		5.58	17.58	3.16		0.36	0.11	0.47	
1	III	8.00	5.26	19.18	3.65	2.25	0.36	0.13	0.39	13.2 gms. pyruvic acid as above.
2	IV		5.74	18.75	3.27		0.29	0.10	0.40	
2	V	7.98	4.47	15.00	3.36		0.19	0.13	0.49	

EXPERIMENT XXV.

Twelve-Hour Periods.

May 1	I	9.31	3.77	12.63	3.35			0.22	1.21	
2	II		3.85	12.10	3.14		0.41	0.39	2.14	
2	III	9.11								
3	IV		3.34	13.80	4.14	5.10	0.23	0.18	0.69	8.8 gms. pyruvic acid as above.
3	V	8.94	3.30	11.60	3.52		0.23	0.35	1.82	
4	VI		4.40	13.10	2.98		0.35	0.60	2.56	

EXPERIMENT XXVI.

Twelve-Hour Periods.

May 16	IV		7.80	28.60	3.68			0.13	0.35	
16	V	12.44	8.35	32.30	3.87	1.16		0.09	0.34	8.8 gms. pyruvic acid as above.
17	VI		7.82	29.51	3.78			0.22	1.00	

SUMMARY.

I. Experiments on phlorhizinized dogs have shown that pyruvic acid is capable of yielding extra glucose in the diabetic organism.

II. In some cases the quantity of glucose was found to be much less than arises from similar amounts of alanine and lactic acid.

III. It is concluded that pyruvic acid cannot be considered a necessary intermediary product in the conversion of alanine into lactic acid and that alanine cannot be considered to undergo oxidative deamination.

SPHINGOMYELIN.

FIRST PAPER. ON THE PRESENCE OF LIGNOCERIC ACID AMONG THE PRODUCTS OF HYDROLYSIS OF SPHINGOMYELIN.*

By P. A. LEVENE.

(From the Laboratories of The Rockefeller Institute for Medical Research,
New York.)

The products of hydrolysis of sphingomyelin have not been the subject of a careful investigation in recent years. The knowledge of the acid components of the substance is limited to the statement of Thudichum¹ that the principal acid taking part in the formation of the molecule is a saturated fatty acid, of the composition $C_{18}H_{36}O_2$, melting at $59^\circ C.$, and hence isomeric with stearic acid. In course of the present investigation it was found that the acid had the composition $C_{24}H_{48}O_2$, a melting point of $81^\circ C.$, and formed an ethyl ester melting at $55-56^\circ C.$, and hence was lignoceric acid. A sample of the ester mixed with that of an ester of lignoceric acid showed the same melting point. The molecular weight of the acid was 368.

EXPERIMENTAL PART.

Sphingomyelin in lots of 40 grams was taken up in 400 cc. of alcohol containing 7 per cent of sulphuric acid and heated with reflux condenser on the water bath for six hours. The product of hydrolysis was allowed to remain at room temperature ($20^\circ C.$) over night, and the ester separated in form of beautiful scales. These were filtered, again taken up in alcohol containing 7 per cent of sulphuric acid, and heated as before for an additional five hours. The ester which separated on the second esterification was filtered off with suction, and recrystallized out of acetone at room

* Received for publication, May 28, 1913.

¹ *A Treatise on the Chemical Constitution of the Brain*, London, 1884.

temperature. Filtered and dried in a vacuum desiccator it had a sharp melting point of 55–56° C. Mixed with a sample of an ester of lignoceric acid obtained on oxidation of cerebronic acid it showed the same melting point of 55–56° C.

0.1428 gram of the substance gave on combustion after Dennstedt 0.4110 gram of CO₂ and 0.1686 gram of H₂O.

	Calculated for C ₂₈ H ₅₆ COOC ₂ H ₅ .	Found.
C	78.80	78.50
H	13.10	13.20

For estimation of the molecular weight the ester was saponified by means of alcohol containing an $\frac{N}{2}$ solution of sodium hydrate.

1.3491 grams of the substance neutralized on boiling for four hours 3.57 cc. of $\frac{N}{2}$ alkali. M. W. = 395.

1.3734 grams of the substance neutralized under the same conditions 3.50 cc. of $\frac{N}{2}$ alkali. M. W. = 391.

1.7848 grams of the substance neutralized 4.55 cc. of alkali. M. W. = 391.

	Calculated for C ₂₈ H ₅₆ O ₂ .	I	Found. II	III
M. W.	368	367	368	363

In order to obtain the free acid the ester was saponified by means of alcoholic sodium hydrate. The soap was decomposed by means of hydrochloric acid, and the free acid repeatedly taken up in water containing hydrochloric acid and warmed on the water bath until the acid melted to an oil. The free acid was finally transformed into the lead salt, and the lead salt decomposed by means of sulphurated hydrogen. The free acid was recrystallized out of toluene. It melted sharply at 81° C., and had the following composition.

0.1160 gram of the substance gave on combustion after Dennstedt 0.3331 gram of CO₂ and 0.1366 gram of H₂O.

	Calculated for C ₂₈ H ₅₆ O ₂ .	Found.
C	78.30	78.33
H	13.00	13.03

ON CHONDROITIN SULPHURIC ACID.

SECOND PAPER.*

BY P. A. LEVENE AND F. B. LA FORGE.

(From the Laboratories of The Rockefeller Institute for Medical Research,
New York.)

It was stated in the previous communication¹ on this subject that chondrosin is composed of two substances, both in some way related to carbohydrates. One was identified as *d*-glucuronic acid; regarding the nature of the other component the views of different writers were most contradictory. Thus, Schmiedeberg² was led to the belief that the substance was glucosamine and Orgler and Neuberg³ announced it to be monoamino-tetrahydroxycaproic acid. Indeed these authors have described crystalline salts of the acid, which were supposedly obtained from the products of barium hydrate hydrolysis of the complex. The most recent reference to the nature of the substance was made by Fränkel,⁴ who regarded it as an amino-glucuronic acid.

Some of the properties of the chondrosin seemed to justify the view of Orgler and Neuberg. Namely, the substance displayed a marked resistance towards boiling mineral acids; and, on the other hand, was readily hydrolyzed by alkalis. For this reason an attempt was made to repeat the experiment of Orgler and Neuberg. Soon, however, it became evident that success could not be expected from these experiments for the reason that even at the low temperature of the thermostat room (40° C.) an aqueous solution of barium hydrate caused chondrosin to give up all its

* Received for publication, May 29, 1913.

¹ This *Journal*, xv, p. 69.

² Schmiedeberg: *Arch. f. exp. Path. u. Pharm.*, xxviii, p. 358, 1891.

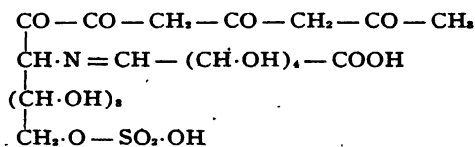
³ Orgler and Neuberg: *Zeitschr. f. physiol. Chem.*, xxxvii, p. 407, 1903.

⁴ S. Fränkel: *Ann. d. Chem.*, cccli, p. 344, 1907.

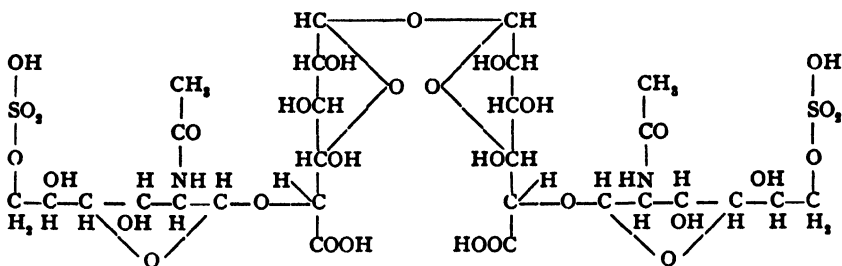
nitrogen in form of ammonia. At this stage of the investigation it was concluded to search for some indirect evidence regarding the nature of the substance, and for this reason an attempt was made to obtain laevulinic acid on treatment of chondroitin sulphuric acid with strong mineral acids. It was shown by Neuberg that glucosamine under certain conditions yields laevulinic acid particularly after preceding deaminization with nitrous acid. Chondrosin was therefore deaminized by means of sodium nitrite and hydrochloric acid, and the product boiled with mineral acids. Two experiments were performed and each yielded small quantities of laevulinic acid. This observation encouraged a renewal of the efforts to obtain glucosamine on direct treatment of chondroitin sulphuric acid with mineral acid. Similar to other workers we met with failures when the experiments were performed on the crude acid sodium salt of chondroitin sulphuric acid. The experiment reported in this communication was performed on a barium salt of chondroitin sulphuric acid free from any mineral impurities. The result of the experiment was most gratifying, as glucosamine hydrochloride was obtained, without an effort, in analytically pure condition.

Thus, for the first time all the components of chondroitin sulphuric acid were isolated and identified. They were those assumed on indirect evidence by Schmiedeberg, namely, sulphuric acid, acetic acid, glucosamine and glucuronic acid.

However, the results of the present investigation necessitate a revision of Schmiedeberg's view regarding the chemical structure of the complex molecule. According to Schmiedeberg's view the structure of the substance is presented by the following graphic formula:



The results of the present investigation lead to the following graphic expression of its composition:



The two views differ in the following. The theory of Schmiedeberg assumes the union of the glucosamine and of glucuronic acid through the formation of a nitrogen bridge. This view is erroneous for the reason that in chondrosin the nitrogen is present in the form of a free amino group, and hence can be removed by nitrous acid. On the basis of the present work one may assume a glucosidic union between the glucosamine and one of the secondary alcohol groups of the glucuronic acid. Of these the γ -position is excluded by virtue of the γ -oxidic structure of glucuronic acid.

The second difference of the two theories relates to the number and place of the acetyl groups entering in the structure of the molecule. Schmiedeberg assumed the presence of three acetyl groups in chondroitin, basing his view exclusively on the percentage composition of the amorphous salts of the substance.

In the course of the present work it was not possible to detect more than one acetyl group on hydrolysis of chondroitin sulphuric acid with mineral acids, or with an aqueous solution of barium hydrate. The presence of only one acetyl group was suggestive on the basis of analytical data, showing in chondroitin sulphuric acid a ratio of N:C=1:14. The same ratio was found by Levene and Jacobs⁵ on the analysis of the barium salt of glycothionic acid obtained from tendo-mucin.

The place of the acetyl group is made obvious by the fact that the nitrogen of chondroitin sulphuric acid is not removed in form of nitrogen gas by nitrous acid, and it was stated already that chondrosin contains its nitrogen in form of an unsubstituted amino group.

⁵ Levene and Jacobs: *Journ. of Exp. Med.*, x, p. 557, 1908.

Finally, the theory expressed here assumes a glucosidic union of two chondrosin molecules, for the reason that this view offers the simplest explanation to the observation that chondroitin does not possess reducing power for Fehling's solution, while chondrosin does.

EXPERIMENTAL.

Laevulinic Acid from Chondrosin.

Fifty grams of chondroitin sulphuric acid barium salt were converted into chondrosin. The solution deaminized with sodium nitrite and evaporated to about 75 cc. in vacuum. Two volumes of 40 per cent hydrochloric acid were then added and the solution boiled for twenty hours. It was then filtered from melanin and extracted several times with ether. The ethereal extract was dried with sodium sulphate, evaporated to dryness and the residue taken up in 250 cc. of water. It contained tin which was removed with hydrogen sulphide and the filtrate, after having been evaporated to remove the latter, was again extracted with ether. The residue from the ethereal solution was taken up in about 2 cc. of water nearly neutralized with ammonia, and the chlorine was removed with a few drops of silver nitrate solution. The addition of concentrated silver nitrate and ammonia to the filtrate produced a white precipitate which was washed with alcohol and ether and dried. The yield was 0.2 gram.

0.1260 gram substance gave 0.0602 gram Ag.

	Calculated for $C_6H_7O_2$	ound.
Ag.	48.24	48.57

Glucosamine Hydrochloride from Chondrosin.

Fifty grams of chondroitin sulphuric acid barium salt were converted into chondrosin, the solution evaporated to a syrup in vacuum and taken up into 150 cc. of water. 150 cc. of concentrated hydrochloric acid were then added together with 10 grams of stannous chloride, and the solution boiled for six hours under a reflux condenser. Without filtering from the separated melanin,

the product was diluted to 500 cc. and the tin removed with hydrogen sulphide. The colorless filtrate from the tin sulphides was concentrated in vacuum to a thick syrup which crystallized spontaneously to a solid cake. This was ground with 95 per cent alcohol in a mortar, filtered and washed repeatedly with alcohol and finally with ether and dried in vacuum. The yield was 9 grams; 2 grams crystallized from the alcoholic mother liquors upon standing.

0.1481 gram of substance gave 17.7 cc. amino nitrogen at 20°, 766 mm.

0.1226 gram substance from alcoholic mother liquors washed and dried in vacuum gave 0.1512 gram CO₂ and 0.0750 gram H₂O.

0.1608 gram of substance recrystallized from 80 per cent alcohol with addition of a few drops of concentrated HCl required 7.72 cc. AgNO₃ solution (1 cc. = 0.00352 Cl).

	Calculated for C ₆ H ₁₃ O ₅ N HCl.	Found.
N	6.51	6.87
Cl	16.45	16.90
C	33.40	33.63
H	6.54	6.79

0.1001 gram substance in 10 cc. H₂O rotated in a 1 dm. tube with D-light + 1°.

$$[\alpha]_{20}^D = 99.9.$$

Nitrogen-Carbon Ratio in Chondroitin Sulphuric Acid Barium Salt.

0.5346 gram of barium salt dried to constant weight in vacuum at 100° gave 8.25 cc. of $\frac{N}{10}$ NH₃.

0.2197 gram of barium salt gave 0.0880 gram H₂O and 0.2110 gram CO₂.

N	2.18 per cent.
H	4.45 per cent.
C	26.18 per cent.

$$N : C = 1 : 14.1$$

Acetic Acid Determinations.

Twenty-five grams of the barium salt of chondroitin sulphuric acid were re-purified by dissolving in 2 liters of distilled water with the addition of 10 grams of pure barium chloride and precipitating by the addition of 1 liter of 95 per cent alcohol. The precipitate was washed chlorine-free with 50 per cent alcohol and finally with absolute alcohol and ether, and dried in vacuum. About 10 grams of this material were dissolved in 10 cc. of water.

The acetic acid determinations were carried out in the following manner. The solution of the chondroitin sulphuric acid barium salt was subjected to simultaneous hydrolysis with 25 per cent sulphuric acid and distillation at ordinary pressure. The distillate was collected in a receiver, cooled with ice and protected from the atmospheric carbon dioxide by soda lime, while the volume of the solution in the reaction flask was kept constant by the addition of water through a dropping funnel.

5 cc. solution gave 7.40 cc. $\frac{N}{10}$ NH_3 (Kjeldahl).
5 cc. solution gave 7.60 cc. $\frac{N}{10}$ acetic acid.
5 cc. solution gave 7.80 cc. $\frac{N}{10}$ acetic acid.
5 cc. solution gave 7.55 cc. $\frac{N}{10}$ acetic acid.

Five cubic centimeters of another solution, which corresponded to 8.50 cc. $\frac{N}{10}$ NH_3 (Kjeldahl) were hydrolyzed in the same apparatus with 20 per cent barium hydrate until no more ammonia was given off. The solution was then acidified with sulphuric acid and the acetic acid determination carried out as above.

5 cc. solution gave 9.75 cc. $\frac{N}{10}$ acetic acid.

ON CEREBRONIC ACID.

THIRD PAPER. ITS BEARING ON THE CONSTITUTION OF LIGNOCERIC ACID.*

By P. A. LEVENE AND C. J. WEST.

(From the Laboratories of The Rockefeller Institute for Medical Research, New York.)

In our study of cerebronic acid¹ an acid of the same composition and melting point as lignoceric acid was obtained by the action of alkaline potassium permanganate upon the potassium salt of cerebronic acid. It was pointed out at the time that this acid might be identical with lignoceric acid. Since then we have prepared derivatives of this new acid, which prove to be the same as those given in the literature for lignoceric acid.²

It has been shown that cerebronic acid has a normal carbon chain by reduction to pentacosan, melting at 53°-54°. This was further confirmed by the isolation of a small amount of an hydrocarbon melting at 51°-52° from the reaction product of hydroiodic acid upon the new acid, C₂₄H₄₈O₂. Thus it is quite definitely established that the new acid has a normal carbon chain. Since this acid is identical with lignoceric acid, then lignoceric must be considered a normal acid of twenty-four carbon atoms.

The following gives a comparison of the acids and their derivatives:

	Lignoceric acid. <i>m. p.</i>	Acid from cerebronic acid. <i>m. p.</i>
Acid	80.5°-81°	80°-81°
Methyl ester	55°	55°
Ethyl ester	57°-58°	58°
Lead salt	117°	116°-117°

* Received for publication, June 9, 1913.

¹ Levene and Jacobs: this *Journal*, xii, p. 381, 1912; Levene and West: *ibid.* xiv, p. 257, 1913.

² Hell and Hermann: *Ber. d. deutsch. chem. Gesellsch.*, xiii, p. 1713, 1880; Kreiling: *ibid.*, xxi, p. 880, 1888.

EXPERIMENTAL.

Methyl Ester.

The methyl ester of the new acid was prepared by boiling 2 grams of the acid, 100 cc. of absolute methyl alcohol and 4 cc. of concentrated sulphuric acid four hours on the water bath. The ester which separated on standing over night at 0° , was recrystallized from ethyl alcohol twice, then from acetone and finally dried in the chloroform bath for two hours. It melted at 58° . Hell and Hermann give 56.5° – 57° ; Kreiling, 58° .

0.1200 gram of substance gave 0.3450 gram CO_2 and 0.1416 gram H_2O .

	Calculated for $\text{C}_{21}\text{H}_{27}\text{O}_5\cdot\text{CH}_3$	Found.
C	78.53	78.41
H	13.09	13.20

Ethyl Ester.

The ethyl ester was prepared in the same way as the methyl ester. It was recrystallized from ethyl alcohol three times, when it melted at 55 – 56° . It was then evaporated to dryness with a slight excess of sodium methylate and extracted with ether to remove any trace of free acid and recrystallized from acetone, when it melted at 55° . Both Hell and Kreiling give 55° as the melting point of the ethyl ester.

0.1194 gram of substance gave 0.3386 gram CO_2 and 0.1408 gram H_2O .

	Calculated for $\text{C}_{23}\text{H}_{29}\text{O}_5\cdot\text{C}_2\text{H}_5$	Found.
C	78.79	79.12
H	13.13	13.20

Lead Salt.

The lead salt was prepared by treating a hot methyl alcohol solution of the acid with lead acetate in the same solvent as long as a precipitate formed. A drop of ammonia was then added to complete the precipitation. This was filtered off when cool, washed with warm methyl alcohol and dried in vacuum. When heated quickly it softened at about 110° and melted at 116° – 117° . Hell and Hermann give the melting point as 117° .

Cerebronic Acid.

0.1200 gram substance gave 0.2694 gram CO_2 and 0.1073 gram H_2O .

	Calculated for $(\text{C}_{24}\text{H}_{37}\text{O}_4)_2\text{Pb}$.	Found.
C	61.19	61.23
H	10.06	10.01

ON THE CEREBROSIDES OF THE BRAIN TISSUE. SECOND PAPER.*

By P. A. LEVENE.

(From the Laboratories of The Rockefeller Institute for Medical Research,
New York.)

In a previous investigation on the "Cerebrosides of the Brain Tissue" Levene and Jacobs¹ have brought to light the following new facts: first, that cerebrosides differ not only in their behavior towards solvents, but also in their optical activity, and second, that fatty acids entering in the molecule of different cerebrosides are not identical. Namely, it was possible to isolate from the product of hydrolysis of mixed cerebrosides the *d*, *l*, and the *dl* forms of cerebronic acid. A small quantity of an acid analyzing for $C_{24}H_{48}O_2$ was also observed. However, it was not obtained in absolutely pure state, its quantity was small, and it was therefore regarded as an impurity.

The cerebrosides employed for last year's work were prepared by the method of Parcus. In this method of preparation fresh, not desiccated, brain pulp is boiled with an aqueous barium hydrate solution. Hence there was a possibility that various optical isomers of the cerebroside isolated in the course of last year's work were artificial products formed in course of preparation. Therefore, it was urgent to establish whether or not brain tissue extracted directly with indifferent solvents contained the same isomers.

In the course of the present year investigations were undertaken on the phosphatides of the brain, and in the process of preparation of this substance, there were obtained considerable quantities of cerebrosides. It was concluded to utilize this material for the investigation of the question whether or not the optical isomers of the

* Received for publication, July 2, 1913.

¹ Levene and Jacobs: this *Journal*, xii, p. 389, 1912.

cerebrosides found in the mixtures obtained by the method of Parcus were of primary or secondary origin.

In the present year's work the material was obtained from desiccated brain tissue. The tissue was dried under diminished pressure, so that complete desiccation took place within very few hours. The dry material was extracted with hot 95 per cent alcohol, each extraction lasting not more than thirty minutes. The deposit formed on cooling of the alcoholic extract served for preparation of cerebrosides.

The material obtained in this manner differed considerably in its general appearance from that obtained by the method of Parcus. The material was separated in a considerable number of fractions, which differed in their solubility.

The fractions also differed in their optical activity. Most of the measurements were made in pyridine solution in a concentration of 6.66 per cent. Under these conditions the fractions were separated into optically active and inactive. The maximum rotation under the given conditions was $[\alpha]_D = +4.14^\circ$. The lowest rotation in pyridine solution was $[\alpha]_D = +3.05^\circ$.

On hydrolysis of the optically active fractions only cerebronic acid was obtained. It consisted, however, of a mixture of the optical isomers.

Since in last year's work the fatty acids were obtained by saponification of the esters by means of alkali it seemed possible that some racemization took place in course of saponification, and that originally the cerebrosides contain only one active acid. It was, therefore, concluded to test directly the optical activity of the esters obtained on hydrolysis of the cerebrosides. In fact it was this object that stimulated the work in the directions of thorough fractionation of the cerebrosides prior to hydrolysis.

It was found that in this direction the work of last year was substantiated, as there were obtained esters varying in their optical activity and having the composition of cerebronic acid ethyl ester. The highest rotation observed was $[\alpha]_D = +2.9^\circ$, the lowest was $[\alpha]_D = +1.6^\circ$. On the other hand, from the more soluble and optically inactive fractions of cerebrosides there were obtained also ethyl esters of cerebronic acid, which were optically inactive. Thus

all these observations coincide with those of last year and seem to justify the conclusion arrived at in the previous communication. These conclusions seem still further justified by the following observation. When the cerebroside with a rotation $[\alpha]_D = +4.14^\circ$, was dissolved according to Thierfelder in chloroform-methyl-alcohol mixture, then the rotation of a 3.6 per cent solution was $[\alpha]_D = +9.5^\circ$, and $[\alpha]_D = +10.7^\circ$ in a 6 per cent solution. Thierfelder's cerebrin had a rotation of $[\alpha]_D = +6.4^\circ - 8.4^\circ$ in a 5 per cent solution, and on hydrolysis yielded only cerebronic acid. Furthermore Thierfelder's kersin gave on hydrolysis 25 per cent of the total fatty acid in form of cerebronic acid and yet it was optically inactive.

Notwithstanding all this, we are inclined to defer decision on the existence of optically isomeric cerebroside containing cerebronic acid until more data on the subject are obtained. The reasons are the following: The more soluble fractions of the cerebroside invariably contain an acid of the composition $C_{24}H_{48}O_2$. In this respect our observations fully coincide with the discovery of Thierfelder,² although the fractionation in our work was carried out by a process totally different from his. Further, two of our samples, which apparently contained only traces of cerebronic acid, and which were optically inactive in a pyridine solution, showed slight dextro-rotation in a Thierfelder's chloroform-methyl-alcohol solution at a temperature just below the boiling point of the solution. Furthermore it was observed that the specific rotation of the cerebroside is considerably lowered by the lowering of the concentration of the tested solution. Hence, decision on the existence of isomeric cerebroside of cerebronic acid has to be deferred until it will be possible to separate completely cerebroside of cerebronic acid from the other cerebroside. The new facts brought forward by the work of Thierfelder and by us carry no definite evidence either against or in favor of our last year's assumption.

Regarding the nature of the acid $C_{24}H_{48}O_2$ our observations differ from those of Thierfelder inasmuch as the melting point of the acid obtained by us, the melting point of its ethyl ester and of its lead salt seemed to coincide with those of lignoceric acid.

² Thierfelder: *Zeitschr. f. physiol. Chem.*, lxxxv, p. 35, 1913.

In this communication only the experiments dealing with the obtaining of the acid $C_{24}H_{48}O_2$ will be described in detail. The methods of fractionation and the properties of the cerebrosides will be communicated when individual cerebrosides are obtained in their final purity.

EXPERIMENTAL PART.

The cerebrine fraction was optically inactive and had the following composition:

0.1139 gram of substance gave on combustion 0.2955 gram of CO_2 , and 0.1187 gram of H_2O . $C = 70.75$; $H = 11.66$ per cent.

0.2000 gram of substance employed for Kjeldahl nitrogen estimation required for neutralization 2.45 c.c. of $\frac{N}{10}$ acid. $N = 1.72$.

The substance contained neither phosphorus nor sulphur.

Ethyl Ester of the Fatty Acid.

Experiment I.—Thirty-six grams of the substance were taken up in 400 cc. of 98 per cent ethyl alcohol to which 20 cc. of sulphuric acid had been added. The mixture was boiled for seven hours with return condenser in a water bath. The reaction product was allowed to remain over night at room temperature of $25^\circ C$. In the morning, the solution was found filled with a mass of glittering scales. These were filtered off on suction. The residue was again taken up in 150 cc. of ethyl alcohol to which 5 cc. of sulphuric acid had been added and the heating repeated as previously for three hours. The reaction product was again allowed to cool at room temperature of $25^\circ C$. over night, and on the following day the mass of glittering scales was again filtered and dried in a desiccator. The weight of this substance was 2.5 grams.

The two mother liquors were combined and placed in a refrigerator at $-1^\circ C$. over night. A second deposit separated in appearance similar to the first. This was again filtered on suction and dried in the desiccator. The weight of the second product was 2.8 grams. The mother liquor from the second deposit was cooled in an ice and alcohol freezing mixture. A gelatinous mass formed which was again filtered on suction and recrystallized out of about 10 cc. of acetone. The sediment formed in acetone was filtered, dried in a desiccator, and amounted to 0.1200 gram.

The first ester was recrystallized out of acetone and dried over night in a vacuum desiccator. It had a melting point of 56–57°C. and the following composition:

0.1116 gram of substance gave on combustion 0.3216 gram of CO_2 and 0.1294 gram of H_2O .

Experiment II.—Twenty-five grams of cerebrine, prepared in the same manner as the substance employed in Experiment I, were taken up in 200 cc. of ethyl alcohol containing 10 cc. of sulphuric acid. This substance was treated in the same manner as in the first experiment. The total yield of the ester was 3 grams. It was not possible to obtain any other esters.

0.1168 gram of substance gave on combustion 0.3356 gram of CO_2 and 0.1398 gram of H_2O .

		Calculated for $\text{C}_{44}\text{H}_{87}\text{O}_2\text{C}_3\text{H}_5$.	Found.
Experiment I	{ C	78.8	78.59
	{ H	13.1	12.89
Experiment II	{ C	78.8	78.49
	{ H	13.1	13.42

A 10 per cent pyridine solution of the ester was found to be optically inactive.

Free Acid.

In order to obtain the free acid the ester was saponified in the usual way by dissolving the ester in ethyl alcohol and adding an excess of aqueous sodium hydrate solution. The solution was boiled with return condenser on the water bath for four hours. The reaction product was poured into acetone, washed repeatedly with acetone, then suspended in ether, shaken vigorously for some time, filtered, and the operation repeated several times. The sodium salt obtained in this manner was decomposed with aqueous hydrochloric acid, and placed on the hot water bath until the free acid melted into an oil. The oil was allowed to solidify, the aqueous hydrochloric acid decanted and the free acid freed from hydrochloric acid by repeatedly suspending it in water, melting it on the water bath and allowing the oil to solidify. When the wash water was free from hydrochloric acid the free lignoceric acid was dissolved in a minimum quantity of absolute ethyl alcohol and the

equivalent of lead acetate dissolved in methyl alcohol was added. A few drops of aqueous ammonia were then added to complete the precipitation of the lead salt. The mixture was allowed to cool, filtered on suction and washed repeatedly with absolute alcohol. The dry product was dissolved in hot toluene and hydrogen sulphide gas was passed through the solution. The filtrate from the lead sulphide was allowed to remain in the refrigerator over night. The acid crystallized out. It was then filtered on suction and the last traces of toluene removed by melting the substance on an electric stove. The melting point of this acid was 81°C . Mixed with a sample of an acid obtained on oxidation of cerebronic acid the mixture gave the same melting point.

Molecular Weight of the Acid.

Experiment I.—0.9566 gram of the acid was dissolved in a mixture of benzin and absolute methyl alcohol and titrated by means of $\frac{N}{2}$ alkali. It required for neutralization 5.2 cc. of the acid.

Experiment II.—1.0626 grams of the substance required for neutralization 5.8 cc. $\frac{N}{2}$ alkali.

	Calculated for $\text{C}_{31}\text{H}_{48}\text{O}_8$.	Found.
M. W. (Experiment I).....	368	368
M. W. (Experiment II).....	368	366

The ethyl ester of lignoceric acid obtained from other fractions of the cerebrosides, which gave on hydrolysis not only lignoceric but also cerebronic acid, showed some irregularity. Thus the ester, when obtained in exactly the same manner as described in the first two experiments, even though it had been re-esterified twice out of acetone, possessed a melting point of between 60 and 62°C . However, when this ester was saponified and transformed into the free acid which had a melting point of 81°C . and a molecular weight of 36, and was again transformed into the ethyl ester, then the melting point came down to 56 – 57°C .

Lead Salt of the Acid.

The lead salt was prepared of the pure acid and recrystallized out of a solution consisting of equal parts by weight of benzene and toluene. The melting point of the substance was at 117.5°C .

*Comparison of the Constants of Lignoceric Acid and of the Acid
Obtained from the Cerebroside.*

	Lignoceric acid.	Acid from cerebroside.
Free acid, M.P.....	80-81°C.	81°C.
Lead salt, M.P.....	117°C.	117.5°C.
Ethyl ester, M.P.....	55°C.	56-57°C.

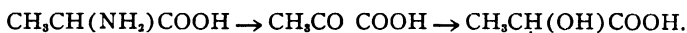
ON THE ACTION OF LEUCOCYTES AND OTHER TISSUES ON *dl*-ALANINE.*

BY P. A. LEVENE AND G. M. MEYER.

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The observations on the action of leucocytes and other tissues under aseptic conditions on hexoses made it possible to disclose the mechanisms of lactic acid formations from glucose.¹ The fact that *d*-lactic acid was formed regardless of the configuration of the hexose employed in the experiments forced the conclusion that methyl glyoxal was the immediate precursor of lactic acid.

It is generally accepted principally on the basis of the work of Otto Neubauer² that the conversion of amino-acids into hydroxy-acids is brought about by a process very analogous to that for formation of lactic acid from methyl glyoxal. Thus, for instance, alanine is transformed into lactic acid by passing through the stage of pyruvic acid.



This contention found support in the observations of Lusk and Ringer.³ These authors fed phlorhizinized dogs on *dl*-alanine and noted a complete conversion of the substance into *d*-glucose. Very recently this view was accepted on theoretical considerations by Dakin and Dudley.⁴ However, in the attempt to corroborate this view experimentally not all writers came to a close agreement. For instance, Paul Mayer,⁵ feeding phlorhizinized dogs on pyruvic acid failed to note a conversion of the substance into glucose. On the

* Received for publication, July 10, 1913.

¹ Levene and Meyer: this *Journal*, xiv, pp. 149, 551, 1913.

² Neubauer: *Deutsch. Arch. f. klin. Med.*, xcv, p. 211, 1909.

³ Ringer and Lusk: *Zeitschr. f. physiol. Chem.*, lxvi, p. 106, 1910.

⁴ Dakin and Dudley: this *Journal*, xv, p. 127, 1913.

⁵ Mayer: *Biochem. Zeitschr.*, xlix, p. 486, 1913.

other hand, Dakin and Dudley, and Ringer,⁶ obtained a positive result. However, they noted that quantitatively the yield of sugar from pyruvic acid was smaller than that from either alanine or from lactic acid. Hence, it was desirable if possible to find a simpler method of testing the theory of the mechanism of lactic acid formation from alanine. Encouraged by the results of the experiments on the action of leucocytes on glucose,⁷ we attempted to test the action of leucocytes on the various optical isomers of alanine. It was thought that if pyruvic acid is the precursor of lactic acid then the same *d*-lactic acid might be obtained regardless of the nature of the alanine employed in the experiment. It was taken for granted that leucocytes possessed the power to deaminize alanine since it is generally accepted that the power of deamination of amino-acids belongs to all tissues. The experiment was begun by the employment of *dl*-alanine. It was a surprise to find that under aseptie conditions leucocytes remained without perceptible action on *dl*-alanine. An attempt was therefore made to test the action of other tissues under aseptie conditions on *dl*-alanine. For this purpose the kidney was employed as previous experience had shown that the kidneys were the most suitable material that could be obtained in absolutely aseptie condition. However, the results of the latter experiments were identical with the results of the leucocyte experiments, namely, they showed that no perceptible deamination took place.

Since these two series of experiments seemed not to harmonize with the generally accepted view on the deaminizing action of the tissues on amino-acids, it was thought that a considerable degree of autolysis of the tissues is required in order to bring their deaminizing action into play. For this reason experiments were performed with tissues in the presence of antiseptics. Kidney and liver were used in the experiments. However, when sufficient antiseptic was added to entirely prevent bacterial contamination no evidence was found of the deamination of alanine in experiments that lasted from one to three weeks.

It is seen from this that alanine is not so readily deaminized

⁶ Ringer: this *Journal*, xv, p. 145, 1913.

⁷ Levene and Meyer: this *Journal*, xi, p. 361, 1912; xii, p. 265, 1912.

as one was inclined to expect on the basis of the work of Lang⁸ and other observers. Whether or not this is true in regard to other amino-acids remains to be established. In a recent article on deamination by Gertrude D. Bostock,⁹ from Cathcart's laboratory, a statement is made that alanine behaves differently from glycocoll on treatment with liver and intestinal mucosa, but the alanine experiments are not reported in detail. We hope to repeat our observations on alanine on a series of other amino-acids.

EXPERIMENTAL.

Tissues.—The kidney and liver used in these experiments were obtained from dogs which had been killed by exsanguination from the carotid while in ether narcosis. The tissues were hashed in a meat chopper and immediately weighed and minced with the alanine and phosphate solutions as described below. The leucocytes were obtained aseptically from the pleural cavity of dogs injected with turpentine according to the method previously described.¹⁰ In one experiment the kidneys of a rabbit removed aseptically were used.

Solutions.—*dl*-Alanine in approximately 2 per cent concentration in a 1 per cent Henderson phosphate mixture was used in all experiments, excepting the leucocyte and aseptic kidney experiment. Toluene was used as antiseptic. The mixtures for each experiment were made up as follows:

Kidney.

1. 20.0 grams tissue suspended in 200 cc. alanine solution.
2. 5.0 grams tissue suspended in 50 cc. alanine solution.
3. 5.0 grams tissue suspended in 50 cc. phosphate solution.
4. 5.0 grams tissue suspended in 50 cc. phosphate solution.

Liver.

1. 40.0 grams tissue suspended in 200 cc. alanine solution.
2. 10.0 grams tissue suspended in 50 cc. alanine solution.
3. 10.0 grams tissue suspended in 50 cc. phosphate solution.
4. 10.0 grams tissue suspended in 50 cc. phosphate solution.

⁸ Lang: *Hofmeister's Beiträge*, v, p. 321, 1904.

⁹ Bostock: *Biochem. Journ.*, vi, p. 48, 1912.

¹⁰ Levene and Meyer: *this Journal*, xi, p. 365, 1912.

Leucocytes.

Approximately equal portions of leucocytes were mixed with

1. 50 cc. alanine solution.
2. 50 cc. alanine solution.
3. 50 cc. phosphate solution.
4. 50 cc. phosphate solution.

In the aseptic kidney experiment only two suspensions were prepared; each minced kidney was suspended in 50 cc. alanine solution. In each instance, suspensions 2 and 4 were coagulated immediately after mixing; 1 and 3 were set aside at 37° for from one to three weeks.

Methods of Analysis.—The contents of each flask were brought to a boil, coagulated and then poured into an excess of absolute alcohol to which was added sufficient 5 per cent alcoholic zinc chloride solution to completely precipitate the protein. This was filtered and the precipitate washed and extracted with hot water, and the filtrate and washing distilled in vacuum to completely remove the alcohol, which was only accomplished by repeating the distillation several times after addition of water. The residue was taken up in water and made up to a definite volume, usually twice that of the original, 50 cc. made up to 100 cc. 10 cc. of this solution were used for a total nitrogen determination, by the Kjeldahl method, 25 cc. for an ammonia determination, and, after removal of the ammonia, for an amino nitrogen determination by the Van Slyke method, as described in detail in a previous communication from this laboratory.

Bacteriological Control.—Aerobic and anaerobic cultures were made of the aseptic mixtures after incubation and smears made of the mixtures with toluene. There was no bacterial contamination. The bacterial examination was made by Dr. H. L. Amoss, and we take this occasion to express our appreciation of his assistance.

Analytical Results.

The amino nitrogen determinations are here reported in detail. The results of the total nitrogen and ammonia nitrogen determinations are included in the appended tables.

EXPERIMENT I.

Kidney Tissue.

	ORIGINAL SOLUTION USED	GAS VOLUME (CORR.)	TEMPER- ATURE	PRESSURE	TIME	N	GRAMS PER 100 CC.
	cc.	cc.	°C.	mm.	min.	mgm.	
Alanine solution:							
Before.....	5	26.20	20	751	4	14.74	0.295
After three weeks.....	5	29.20	24	758	4	16.25	0.325
Phosphate solution:							
Before.....	5	1.30	24	760	4	0.78	0.015
After three weeks.....	5	3.30	24	758	4	2.00	0.040

EXPERIMENT II.

Kidney Tissue.

	ORIGINAL SOLUTION USED	GAS VOLUME (CORR.)	TEMPER- ATURE	PRESSURE	TIME	N	GRAMS PER 100 CC.
	cc.	cc.	°C.	mm.	min.	mgm.	
Alanine solution:							
Before.....	5	28.00	18	760	4	16.10	0.322
After one week.....	5	30.30	20	758	4	17.30	0.346
Phosphate solution:							
Before.....	5	0.70	18	766	4	0.40	0.008
After one week.....	5	2.80	20	758	4	1.40	0.028

EXPERIMENT III.

Liver Tissue.

Alanine solution:							
Before.....	5	27.90	24	760	4	15.60	0.310
After three weeks.....	5	32.20	24	758	4	18.10	0.362
Phosphate solution:							
Before.....	5	2.20	24	760	4	1.28	0.026
After three weeks.....	5	6.20	24	758	4	3.44	0.068

EXPERIMENT IV.

Liver Tissue.

Alanine solution:							
Before.....	5	27.80	18	760	4	16.10	0.322
After one week.....	5	29.10	20	758	4	16.50	0.330
Phosphate solution:							
Before.....	5	1.00	18	766	4	0.58	0.012
After one week.....	5	3.30	20	758	4	1.87	0.037

EXPERIMENT V.

Leucocytes.

Alanine solution:							
Before.....	2.5	14.75	22	760	4	8.31	0.332
After three weeks.....	2.5	17.60	20	758	4	19.90	0.398
Phosphate solution:							
Before.....	2.5	0.20	22	760	4	0.113	0.004
After three weeks.....	2.5	3.20	20	758	4	1.81	0.073

EXPERIMENT VI.

Aseptic Kidney.

Alanine solution:							
Before.....	2	11.70	20	760	4	6.65	0.332
After three days..	2	11.70	20	758	4	6.64	0.332

EXPERIMENTS WITH KIDNEY TISSUE AND ALANINE.
WITH TOLUENE.

	AMMONIA N PER CENT			AMINO N PER CENT			TOTAL N PER CENT		
	Before	After	Diff.	Before	After	Diff.	Before	After	Diff.
I. Three weeks' incubation:									
Tissue and alanine.....	0.003	0.008	0.005	0.205	0.325	0.030	0.350	0.400	0.050
Tissue and phosphate solution...	0.003	0.006	0.003	0.015	0.040	0.025	0.056	0.100	0.044
II. One-week incubation:									
Tissue and alanine.....	0.004	0.006	0.002	0.322	0.346	0.024	0.336	0.350	0.014
Tissue and phosphate solution....	0.006	0.010	0.004	0.008	0.028	0.020	0.0084	0.0137	0.005

EXPERIMENTS WITH LIVER TISSUE AND ALANINE.

III. Three weeks' incubation:									
Tissue and alanine.....	0.003	0.012	0.008	0.310	0.362	0.052	0.346	0.455	0.109
Tissue and phosphate solution...	0.005	0.011	0.006	0.026	0.068	0.042	0.045	0.178	0.133
IV. One-week incubation:									
Tissue and alanine.....	0.006	0.010	0.004	0.322	0.330	0.008	0.370	0.434	0.064
Tissue and phosphate solution....	0.006	0.013	0.007	0.012	0.037	0.015	0.053	0.128	0.075

EXPERIMENTS WITH LEUCOCYTES.
WITHOUT ANTISEPTICS.

V. Three weeks' incubation:									
Leucocytes and alanine.....	0	0.014	0.014	0.332	0.398	0.066	0.384	0.520	0.136
Leucocytes and phosphate solution.....	0	0.007	0.007	0.004	0.073	0.067	0.056	0.192	0.136

NOTE ON A CASE OF PENTOSURIA.*

By P. A. LEVENE AND F. B. LA FORGE.

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The presence of pentose in urine was first demonstrated by Salkowski¹ in 1892. Later Neuberg² undertook an investigation into the nature of the pentose. He came to the conclusion that the pentose was *dl*-arabinose. Numerous observers corroborated Neuberg's statement, inasmuch as they found that urine showing the presence of pentose, as a rule, was optically inactive. Only a few instances are recorded in which the urine containing pentose showed optical activity. In the few cases recorded by Luzzatto,³ Blumenthal,⁴ and by Schüler,⁵ the urines were dextro-rotatory. Blumenthal and Schüler have taken for granted that the sugar present in the urine was arabinose and hence regarded the pentose observed by them as *l*-arabinose. Luzzatto prepared the phenyl osazone of the pentose present in the urine of his patient and observed a rotation of $+1.04^{\circ}$ in a concentration which required according to the theory a rotation of $+1.10^{\circ}$. On the basis of this he came to the conclusion that the sugar was *l*-arabinose.

Very recently Elliott and Raper⁶ reported on a case of pentosuria in which the sugar failed to form derivatives with diphenyl hydrazine, nor did they succeed in obtaining any other evidence that the sugar was arabinose, and in fact suggested the possibility that the sugar was ribose.

A few years ago a urine containing a pentose came to our obser-

* Received for publication, July 10, 1913.

¹ Salkowski and Jastrowitz: *Centralbl. f. d. med. Wiss.*, xxx, p. 337, 1892.

² Neuberg: *Ber. d. deutsch. chem. Gesellsch.*, xxxiii, p. 2243, 1900.

³ Luzzatto: *Hofmeister's Beiträge*, vi, p. 87, 1905.

⁴ Blumenthal: *Realensycklopädie*, xxxii, p. 388, 1908.

⁵ Schüler: *Berl. klin. Wochenschr.*, xlvii, p. 1322, 1910.

⁶ Elliott and Raper: *this Journal*, xi, p. 211, 1912.

vation. A phenyl osazone prepared from the sugar showed dextro-rotation of $+0.86^\circ$ in a concentration and in a solution prepared according to the directions of Neuberg. Under such conditions *l*-arabinose shows a rotation of $+1.10^\circ$, and *l*-xylose a rotation of $+0.15^\circ$. Hence it was evident that the pentose present in the urine under our observation belonged to the arabinose group. In fact, very frequently in course of the work on nucleic acid phenyl osazone of *d*-ribose was obtained which in the same concentration and in the same solution as used in the present experiment showed an identical rotation of -0.86° . An attempt was therefore made to establish the nature of the pentose. Great difficulties, however, were encountered in the attempt to obtain the sugar in a higher concentration. The urine contained only 0.25 per cent of the pentose (calculated on the basis of glucose). At first an attempt was made to concentrate the urine at a pressure of about 18 mm. and a temperature of about 40° . However, when the urine was concentrated to a thick syrup, it gave only a slight test with orcin and possessed only insignificant reducing power for Fehling's solution. Evidently the greater part of the pentose was either destroyed or had undergone some condensation so that the usual derivatives of it were no longer obtainable.

It is worthy of note that Luzzatto had already called attention to the fact that the concentrating of the urine of his patient led to a considerable loss of the pentose. It was necessary to find a way by which the sugar could be obtained in greater concentration than present in the urine before any more detailed investigation into the nature of the pentose could be undertaken. It was found that the addition of weak acids or of barium carbonate to the urine to some extent improved the condition so that the pentose could be obtained in concentration of 1 to 2 per cent. But, even then, the concentrated urine showed the evidence of considerable decomposition of the pentose.

An attempt was then made, first to remove the urea of the urine and then to obtain the pentose by means of a solution of lead acetate and of barium hydrate. The lead precipitate of the pentose was then decomposed in the usual way and the aqueous solution containing the sugar concentrated under diminished pressure and at a

low temperature. Solutions obtained in that manner contained very little nitrogen but at the end of concentration again showed a considerable destruction of the pentose. Also this method, therefore, was abandoned. Finally it was concluded to dry the lead precipitate of the sugar and then to extract it with alcohol containing just sufficient hydrochloric acid to remove the lead. In this manner an alcoholic solution of the pentose was obtained. It was hoped that this alcoholic solution of the pentose could be used directly for the preparation of the various hydrazones. However, in no instance was it possible to obtain a diphenyl hydrazone characteristic for arabinose. Even in solutions which contained the pentose in a fairly high concentration an insoluble hydrazone could not be obtained. On the other hand, the addition of arabinose either directly to the urine, or to the concentrated urine, or to the solutions obtained on decomposition of the lead derivatives always led to the formation of the hydrazone. The failure to obtain the diphenyl hydrazone naturally can be interpreted in two ways: first, that the urine contained some substances which prevented the hydrazone from crystallizing; and second, that the pentose was not arabinose, but either ribose or keto-arabinose. It is worthy of note that the residue obtained on evaporation of the alcoholic extract from the lead compound of the sugar contained a large proportion of organic non-nitrogenous material which was not the pentose. It is undoubtedly due to the presence of these substances that a crystalline hydrazone of the pentose was not obtainable. It was also impossible to obtain in crystalline form an oxidation product of the pentose.

In order to obtain some information into the nature of the pentose the optical rotation of the sugar solution was compared with its reducing power for Fehling's solution. In this manner it was found that the specific rotation of the sugar was $+17.5^{\circ}$. The specific rotation of *l*-arabinose is $+104.4^{\circ}$ and that of ribose is $+19.3^{\circ}$. On the basis of this it seems more probable that the pentose present in the urine under our observation is not *l*-arabinose but *l*-ribose. We fully realize, however, that the exact nature of the sugar cannot be made certain before it is obtained in a purity that will permit the obtaining of some well-defined derivatives.

This report is made at this stage for the reasons, that on the one hand the material is not accessible for further work, and second that it suggests the possibility that there may exist more than one form of pentosuria.

EXPERIMENTAL.

Osazone.

400 cc. pentose urine were heated on the water bath with 5 grams of phenyl hydrazine. The osazone began to separate almost at once and after one hour the crystals were filtered off and recrystallized from water containing pyridine. It melted at 163° C.

0.1264 gram substance gave 0.0712 gram H₂O and 0.2872 gram CO₂.

	Calculated for C ₁₇ H ₂₀ O ₅ N ₄ .	Found.
C	62.19	61.98
H	6.10	6.24

0.1002 gram osazone in 5 cc. pyridine alcohol rotated in a 0.5 dm. tube with D-light + 0.43°.

5 liters of urine were precipitated with mercuric acetate and sodium hydrate. The mercury was removed from the filtrate with hydrogen sulphide and a small amount of lead acetate solution added to remove the excess of the latter. The filtrate was then treated alternately with a solution of basic lead acetate and barium hydrate until no further precipitate was produced. The lead and barium precipitate was washed with water and decomposed by shaking with 5 per cent. sulphuric acid. The excess of the sulphuric acid was removed with barium carbonate and the amount of sugar calculated as glucose found by titration to be 3 grams. The solution could not be concentrated without destruction of the sugar.

1 PER CENT RIBOSE SOLUTION	FEHLING'S SOLUTION	N 10 SULPHOCYANATE	FOR 1 CC.
cc.	cc.	cc.	
2	14	4.70	2.35
3	21	8.30	2.76
4	28	10.40	2.60
4	28	10.60	2.65
5	35	13.30	2.66
7	49	18.50	2.60

2 cc. of solution corresponded to 13.5 cc. ammonium sulphocyanate (1 cc. ^N/₁₀ ammonium sulphocyanate = 377 mgm. ribose).⁷ Hence, 100 cc. contained 2.54

⁷ The exact data regarding the reducing power of ribose for Fehling's solution are as yet lacking. The following experiments were made principally to obtain the factor required for the present work. More detailed and numerous determinations are still needed. The reducing power of ribose was first determined approximately by varying the amount of Fehling's solution required to reduce a given amount of 1 per cent solution, and the following determinations carried out by Volhard's method, using the required volume of the same concentration of Fehling's solution.

grams calculated from the absolute reducing power of ribose. It rotated in a 2 dm. tube with D-light $+0.91^{\circ}$.

$$[\alpha]_D^{20} = +17.5^{\circ}.$$

Concentration of the Pentose.

By extracting the dried precipitate from the lead and barium with warm alcoholic hydrochloric acid a solution of the pentose was obtained, which after removal of the hydrochloric acid with an excess of lead carbonate could be concentrated to a syrup which is free from nitrogen. However, the total weight of the residue exceeded enormously the calculated weight of sugar in it.

Attempts to Prepare Hydrazones of the Pentose.

20 cc. of a solution containing 0.4 gram sugar were treated with 0.5 gram parabromophenyl hydrazine and the solution concentrated in vacuum at room temperature in a desiccator. A syrup was left which did not crystallize.

The experiment was performed with benzyl phenyl hydrazine with addition of an equal volume of alcohol and boiling for one hour on the water bath. Upon concentrating in vacuum only a syrup was obtained which did not crystallize.

The same experiment with diphenyl hydrazine also led to a negative result. The last two experiments were repeated. The addition of 0.4 gram of arabinose yielded the respective hydrazones.

5.6 grams sugar in 750 cc. of water were treated with 3.9 grams (1 mol.) phenyl hydrazine and the solution evaporated at low temperature in vacuum. Instead of a hydrazone the above described osazone was obtained.

CONCERNING VISCERAL ORGANISMS.*

By ALEXIS CARREL, M.D.

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New York.)

In previous articles¹ it has been shown that a fragment of heart pulsates normally for more than 100 days after its extirpation from the animal, and that connective tissue can be kept indefinitely proliferating *in vitro*. We have now observed strains of connective tissue cells that are still multiplying actively after more than sixteen months of life outside the organism. But up to the present only fragments of tissue have survived *in vitro*. Since the survival of entire organs outside of the body would undoubtedly have important physiological uses, I began in June, 1912, to develop a technique by means of which a system of organs could be made to live and functionate when separated from the other organs. Within a few months this technique has been sufficiently perfected to permit the separated organs being put to practical use for the study of several problems.

The method consists in removing aseptically the abdominal and thoracic organs of an animal and in preserving the organs in an incubator at a temperature of 38° C., while the lungs are being artificially ventilated. The operations were performed on both cats and dogs, but more often on cats. The animals were etherized and killed while they were still under the influence of the ether. Aseptic technique was employed and the same general rules were observed as in complicated thoracic or abdominal operations.

Two animals were used for each operation; the first one was prepared for transfusion, and from the second the viscera were taken.

* Received for publication, May 15, 1913.

¹ Carrel, A., *Jour. Exper. Med.*, 1912, xv, 516; Carrel, A., and Burrows, M. T., in Abderhalden, E., *Handbuch der biochemischen Arbeitsmethoden*, 1912, pt. v, 836; Ebeling, A. H., *Jour. Exper. Med.*, 1913, xvii, 273.

The first animal was etherized, the hair on the anterior part of the neck was removed with sodium sulphide, and the skin was washed with alcohol and sterilized with iodine. The neck was opened by longitudinal incision and one of the carotid arteries was dissected and opened after the circulation had been interrupted by a *serre fine*. The blood was washed out from the lumen of the vessel with Ringer solution, and the end of a glass cannula about thirty centimeters long was introduced and fixed by means of a circular ligature into the lumen of the artery. The cannula was not paraffined and no oil was used, on account of the danger of fat embolism of the coronary arteries of the second animal. Then the animal was kept etherized until it was time to transfuse the blood to the second animal.

The second animal was etherized, and the skin on the anterior part of the neck, thorax, and abdomen was shaved and sterilized by means of the technique previously described. The operation comprised four stages.

1. A long incision was made in the middle line from the upper part of the neck to the lower part of the abdomen, and the skin was dissected on each side of the line of incision. The trachea was dissected, and the esophagus was isolated, ligated, and cut under aseptic precautions. In some cases a catheter was introduced into the esophagus as far as the stomach, and fixed in such a way as to prevent any infection. Then the trachea was cut transversely and a curved glass cannula was inserted into its lumen and fixed by two circular ligatures. A gum catheter was introduced into the glass cannula and the trachea, as far as the bifurcation, according to the Meltzer-Auer method. The end of the catheter was connected with a foot bellows or a small electric blower.

2. The abdominal wall was opened on the middle line from the sternum to the pubis. The abdominal viscera were wrapped in a Japanese silk towel sterilized in vaselin, and were pushed up into the upper part of the abdomen. Branches of the mesenteric artery were ligated and cut, and the small intestine was prepared for section at a short distance from the large intestine. Then it was severed after having been ligated, and was cauterized with carbolic acid. The aorta and vena cava were exposed near the point of their bifur-

cation, ligated, and cut. The ureteral vessels were ligated and the ureters were severed. When the bladder had to be kept the ureters and their vessels were dissected as far as the posterior part of the bladder. The bladder itself was isolated from the surrounding tissues, the urethra was cut, after having been ligated, and the bladder was raised to the level of the kidneys. Then the aorta and vena cava were isolated from the abdominal wall and their posterior branches were tied. The peritoneum surrounding the kidneys was dissected and cut. All the small vessels uniting the aorta and the vena cava to the posterior part of the abdomen were also ligated and cut. The splanchnic nerves were cut and also the pillars of the diaphragm. Then the abdominal viscera, being completely separated from the abdominal wall, were wrapped in a Japanese silk towel. They remained united to the animal by the aorta and vena cava and their circulation was still active.

3. A transverse incision was made in the third or fourth intercostal space. The mammary arteries were clamped. Then the costal cartilages were cut on the right side of the sternum, and the thorax was widely opened. The diaphragm was severed at a short distance from the thoracic wall, and artificial respiration was established. It consisted of a current of air introduced into the trachea by means of a catheter and interrupted about sixteen times every minute. The lungs and the heart were protected by a Japanese silk towel, impregnated with vaselin. Then the innominate arteries or the carotid arteries were ligated and cut. The superior vena cava and the azygos vein were also tied and cut. After a few minutes the animal died, but the heart continued to pulsate. The vagus, sympathetic, and phrenic nerves were severed. Then the pleurae were cut on each side of the thoracic aorta. All the posterior branches of the thoracic aorta were clamped by means of a small Gentile hemostatic forceps and cut close to the thoracic wall. Generally the heart pulsated weakly and the blood pressure was low. The lungs and the abdominal organs were pale and no pulsations could be seen in the small arteries. In some experiments the heart stopped pulsating entirely.

4. Then the thorax and abdominal viscera, united by their blood vessels, were removed from the cadaver of the animal and placed

in a tray containing Ringer solution at a temperature of about 38° C., in such a manner that the lungs floated on the surface of the fluid and the heart was suspended underneath in the liquid. When this position was reversed and the heart was placed above the lungs the circulation was somewhat impaired. The temperature of the Ringer solution was maintained constant by means of an electric pad placed under the tray, or simply by the addition from time to time of Ringer solution at the right temperature. Ordinarily the heart still pulsated slowly and regularly, but the blood pressure was low and the appearance of the organs anemic. After a few minutes the blood pressure began to rise, and in a few cases became almost normal. Generally it remained low and sometimes the heart entirely ceased beating. Then a transfusion was made from the carotid artery of the first animal to the inferior vena cava or abdominal aorta of the visceral organism. When the pressure was very low or when the pulsations of the heart had completely stopped, the transfusion was made directly through the aorta, in order to reestablish immediately a normal circulation through the coronary arteries. The heart started almost immediately to pulsate normally. As the condition of the heart improved the transfusion was made through the inferior vena cava. It was possible in that way to inject quickly a large quantity of blood into the visceral organism. Immediately after the transfusion the lungs became pink, the heart beat strongly from 120 to 150 times a minute, and the blood pressure often rose above normal. The abdominal aorta pulsated violently and strong pulsations could be seen in the arteries of the stomach, liver, kidney, intestine, and even of the ovaries. Peristaltic contractions of the stomach and of the intestines were observed. The spleen, which was bluish, assumed its normal appearance. After a few minutes all the viscera were apparently normal. Then a careful hemostasis of the posterior branches of the thoracic aorta was made and all the forceps were removed. It was important to ascertain that no hemorrhage, even one from a very small vessel, was taking place. If a few minutes after the transfusion the pressure was still above normal, a certain quantity of blood was allowed to flow from the lower part of the abdominal aorta. Then the appearance of the viscera exactly resembled that of a normal animal.

5. The visceral organism was placed in a tin box filled with Ringer solution, and covered with Japanese silk and protected by a glass cover. The tracheal and esophageal tubes were fastened to proper openings in the anterior part of the box. The intestine was pulled through a glass and rubber tube fixed in the posterior wall of the box. The end of the intestine was fixed by circular suture to the edge of the rubber tube, an artificial anus being made. The box was put into an incubator at a constant temperature of about 38° C. Artificial respiration was carried on by a current of air interrupted about twelve times a minute. The compressed air was furnished by means of an automatic electric apparatus pumping air into a tank, from which it was given to the animal under the proper pressure. Dr. Meltzer kindly examined the respiratory conditions in these experiments and showed me how a proper ventilation of the lungs could be obtained. His advice contributed in a large measure to the rapid success of the work. Water or food could be injected into the stomach through the esophageal tube. The urine could be collected from the bladder through a tube, but it was generally aspirated from the bladder with a needle, when samples of urine were taken for study. The feces and intestinal secretions were received outside of the box from the artificial anus.

It was then observed that during the hours following the operation the viscera had the same appearance as those of a living animal. The contractions of the heart and the circulation of the organs were apparently normal. Pulsations could be seen in the smaller branches of the mesenteric artery. The intestine emptied itself through the artificial anus by means of regular peristaltic contractions. When the intestine was empty, bile and intestinal juices were evacuated. In an experiment in which the stomach was full of meat at the time of death, digestion took place. Dr. Van Slyke found that at least 90 per cent. of the amino acids injected into the intestine were rapidly absorbed. There was also an abundant secretion of urine, which was collected into the bladder. After five or six hours hyperemia of the peritoneum of the intestine appeared. It seemed as though a peritonitis developed progressively, and in some cases the intestines became paralyzed after eight or nine hours, although their

circulation was still very active. Abundant hemorrhage could still be produced by section of a small branch of the mesenteric artery. Some of the visceral organisms died almost suddenly after three or four hours, but most of them were in a normal condition ten and even twelve hours after the death of the animal to which the organs belonged. The death of the organism was announced by some irregularities in the pulsation of the heart, which was also weaker. Then the heart stopped suddenly. In one experiment the death of the visceral organism occurred thirteen and one quarter hours after the death of the cat from which it was taken.

The technique of the operation can be modified in many different ways. The number and the nature of the viscera composing the organism were varied. The intestines, spleen, or kidney can easily be removed. It is also possible to exclude the liver from the circulation by ligation of the portal vein after a lateral anastomosis between the portal vein and the vena cava. The thyroid gland can be kept with its circulation or removed completely. The composition of the organism should be adapted to the problems which are to be studied.

The technique of preserving the organism is far from being definitely settled. The organs were generally kept in Ringer solution. Other kinds of fluid, such as serum or ascitic fluid, can also be used. I attempted to keep the viscera without any fluid at all in fine Japanese silk towels impregnated with vaselin. In this case the organism was put into a bag of very fine rubber, open at each end. Through one end was fixed the tracheal tube and to the other end the anal tube. The sac was then suspended in water. There was less danger of infection, but so far the results have not been as satisfactory as when the organs were placed directly in Ringer solution. In another experiment the entire thoracic cavity was kept. The operation consisted merely of a double amputation, through the abdomen and through the neck. A circular incision was made around the abdomen and the skin was dissected. The abdominal cavity was opened, and the intestine, aorta, and vena cava were ligated and cut. Then the anterior wall of the abdomen was sutured to the posterior wall and the abdominal cavity completely closed. The spinal column was cut

and the skin sutured. Afterwards the head was amputated and the stump was covered with the skin, leaving only an opening for the tracheal tube. A transfusion was made through the lower part of the aorta and the heart went on pulsating almost normally. Then the reduced organism was put into an incubator. It died of emphysema, because the air pressure became too high and produced rupture of the lung.

The technique will probably be progressively modified and adapted to the various problems of pathology, physiology, and biological chemistry, for the study of which the visceral organisms can be used.

CONTRIBUTIONS TO THE STUDY OF THE MECHANISM OF THE GROWTH OF CONNECTIVE TISSUE.*

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New York.)

PLATES 10 TO 18.

In previous articles¹ it has been shown that connective tissue cells can be preserved permanently *in vitro* in a condition of active life. Strains of these cells have now been proliferating rapidly outside of the organism for more than sixteen months. In the course of experiment it was observed that a constant relation existed between the rate of growth and the composition of the medium. This fact indicated that certain cell phenomena of the higher animals, such as multiplication, growth, and senility, might now be investigated profitably. Since the time of Claude Bernard it has been known that the life of an organism is the result of the interactions of the cells of which it is composed and of their *milieu intérieur*. But the nature of the interactions has not yet been ascertained; for in order to discover the laws by which they are regulated it would be necessary to modify the humors of the organism and to study the effect of these modifications on the growth of the tissues. This could not be done on account of the lack of a proper method; but this investigation is now rendered possible because of a technique which permits strains of connective tissue cells to multiply indefinitely *in vitro*, like microorganisms.

I have attempted to ascertain some of the relations which exist between the tissues and their medium, by examining the way in which the medium reacts upon the dynamic condition of the cells

* Received for publication, June 4, 1913.

¹ Carrel, A., *Jour. Exper. Med.*, 1912, xv, 516; Pozzi, F., *Bull. de l'Acad. de méd.*, 1912, lxxvii, 475; Ebeling, A. H., *Jour. Exper. Med.*, 1913, xvii, 273.

of a tissue, and how, in turn, the cells modify the properties of the medium. Nearly all the experiments were carried on during the first months of 1913 with strains of connective tissue cells derived from tissues extirpated from embryonic or adult chickens during the months of January and June, 1912, and February, 1913. The fragments of tissue composed of these cells were preserved in a medium composed of two parts of adult chicken plasma and one part of juice taken from a chick embryo eight days old. Every two or three days the tissue was removed from the culture medium, washed for thirty seconds or one minute in Ringer solution, divided into two or three parts if its volume had increased, and placed in new medium at a temperature of 39° C. This fragment soon surrounded itself with a large number of cells, which formed a new tissue. After forty-eight hours the ring of new tissue was measured with a micrometer. Its width was usually 76.5 or 91.8 microns. The rate of growth remained constant as long as the composition of the culture medium was not modified.

THE INFLUENCE OF THE MEDIUM ON THE ACTIVITY OF THE CELLS.

The effect of the medium upon the rate of proliferation of the cells was studied in two series of experiments. In the first series fragments of tissue composed of cells of known activity were placed in media of different composition. In the second series strains of cells of varied conditions of activity were placed in media of the same composition. By measuring the growth of the fragments of tissue which had been living under these different conditions for several generations it was possible to ascertain in what manner the media affected the development of the tissue.

Series 1.—In the first experiments the influence of different media was studied. Normal adult chicken plasma was the basis of the medium but it was changed in many different ways by increasing or diminishing its osmotic tension, by diluting it, by modifying its reaction, and by adding certain organic or inorganic substances to it. The rate of growth of a given strain of cells cultivated in the different media varied according to their composition. The effect of a given medium was ascertained only when the tissues had been developing in it for several generations.

The influence on tissues of normal adult chicken plasma was first studied. It was found that the rate of development of a tissue cultivated in plasma alone varied according to certain conditions of the animals from which the plasma was taken. The plasma of some chickens was more activating than that of others, and the greatest difference in the results was caused by the varying ages of the animals,—the younger the animal that supplied the plasma the greater the growth of the tissue. Quantitative experiments were made by employing plasma taken from chickens aged four and five months and aged five and six years. It was found that after three days the size of the ring of new tissue, in the plasma of an old animal, was 30.6 to 38.25 microns, whereas in the plasma of a young animal, it was 45.9 or 53.55 microns. When the plasma of an adult animal, whether chicken or dog, was used, the connective tissue lived for several weeks, but the mass of tissue did not increase. In the first experiments, which were performed in October, 1911, in connection with the rejuvenation of cultures, the fragments of tissue were cultivated in the plasma of an adult animal.² The increase in volume was so slight that the loss of cells caused by the section of the medium and the passages deprived the culture of nearly all the new tissue formed during its period of active life. After fifty-five days the fragments of connective tissue which had been growing constantly were no greater than on the first day. This proved conclusively that the plasma of an adult animal did not possess the power of producing a large increase of the mass of the tissue. It was next attempted, by modifying the conditions of the medium, to impart to the plasma of an adult animal a dynamic power analogous or superior to that possessed by the plasma of a young animal. It was found that in a medium composed of plasma and of embryonic juice the volume of tissue increased with great rapidity. The tissue grew so much that it became necessary to divide and subdivide it. Thus a large number of new cultures were made from tissues derived from the few fragments extirpated in January and June, 1912. This activating power is not peculiar to embryonic juice, for extracts taken from

² Carrel, A., *Jour. Am. Med. Assn.*, 1911, lvii, 1611.

most of the organs of an adult animal possess this property in a lesser degree. When an animal is old, the juice becomes less activating. Whereas the muscle extract of a young chicken produced in two days a ring of new tissue 76.5 or 91.8 microns in width, that of an old fowl produced a ring of only 22.95 microns.

The action of media of different composition on a given strain of cells was studied in a great many experiments. A fragment of tissue composed of cells which had been living for several months *in vitro* was divided into two equal parts. One part was cultivated in normal plasma and the other in plasma containing different quantities of embryonic juice or other substances. The difference in the quantity of tissue produced in forty-eight hours around the fragments cultivated in plasma alone and mixed with embryonic juice was very large (figures 1 and 2). After several passages the growth of the tissue living in normal plasma became progressively reduced and often stopped completely. On the other hand, the growth of the tissue cultivated in a mixture of plasma and embryonic juice was so abundant that it had to be divided repeatedly. This is shown by experiments of which the following is a type.

The tissue contained in culture 5,142, which had undergone 150 passages and which was growing rapidly and regularly, was divided into two parts, A_1 and B_1 . Part A_1 was placed in adult chicken plasma, while part B_1 was cultivated in a medium composed of two parts of plasma and one part of embryonic juice. After forty-eight hours the width of the ring of new tissue formed around A_1 was 30.6 microns, while around B_1 it was 91.8 microns (figure 3). A second time A_2 was cultivated in normal plasma and B_2 in plasma and embryonic juice. After forty-eight hours B_2 was surrounded by a ring 91.8 microns in width, while not a single new cell could be seen around A_2 (figure 4). Nevertheless, the tissue of A_2 was not dead, and it started to grow when it was replaced in a mixture of plasma and embryonic juice (figures 5 and 6). By increasing or diminishing the proportion of embryonic juice contained in normal plasma it became possible to regulate the quantity of new tissue produced in forty-eight hours by a group of connective tissue cells. After a few passages a strain of cells cultivated in adult chicken plasma containing a given quantity of embryonic

juice was growing at a constant rate. After more than sixteen months the strains of connective tissue cells were proliferating at the same rate as the original piece of embryonic tissue from which they were taken. Thus it was demonstrated that connective tissue which had been living *in vitro* for more than one year could greatly increase its mass in a short time. A fragment of tissue composed of the strain of cells of January, 1912, which had undergone 130 passages, was photographed (figure 7), then divided, and photographed again (figure 8) after forty-eight hours. These photographs show that the volume of the tissue had at least doubled during these two days. In the experiments performed with the strain of June, 1912, which had undergone eighty-seven passages, the increase in volume was almost identical. A fragment of tissue composed of these cells was photographed (figure 9), and after being divided was transferred to a new medium. Then infection occurred and the bacterial colony was extirpated together with about one fourth of the tissue; but the remaining part proliferated actively and the tissue was divided and subdivided. Other photographs (figures 10, 11, and 12) were taken during the following days. By comparing the first photograph and the photographs taken on the fourth, sixth, and tenth days it is possible to see what enormous quantities of cells were produced from the small original fragments. In ten days the volume of the tissue had increased at least fifteen times. In other experiments fragments of tissue derived from the strain of cells which had lived for more than fourteen months outside of the organism were cut in smaller pieces and suspended in Ringer solution. A drop of the suspension mixed with normal plasma and embryonic juice was placed in various test-tubes. After two days a sphere composed of elongated connective tissue cells had developed around each small fragment (figure 13). When two spheres were sufficiently close they became united by a bridge composed of chains of active tissue cells (figure 13). The increase of the mass was very large, and in one experiment the volume of the tissue augmented more than fifty times in six days. The experiments show the importance that is attached to the composition of the culture medium, since the growth of a strain of cells, the volume of which increased so rapidly in a

proper medium, remained stationary when it was merely cultivated in normal adult chicken plasma. As the rapidity of growth of a tissue depends so much on the composition of the medium, it may become possible to use as a reagent of the dynamic value of the humors of an organism a strain of cells adapted to life *in vitro*. If human connective tissue cells could be preserved in a condition of permanent life, as connective tissue cells of the chicken are preserved, the value of the plasma of an individual might be appreciated by the cultivation in it of a group of these cells and by the observation of the rate of their multiplication. These observations would perhaps give some indication of certain values of the blood of an organism and possibly some clew to its age.

Series 2.—In the second series of experiments, strains of cells of varied activity were cultivated in identical media. Modifications in the rate of growth occurred and the progressive changes of the dynamic condition of the cells under the influence of the medium could be studied. It is known that the dynamic state of different tissues taken from a normal animal, or that of tissues previously cultivated *in vitro* under different conditions, varies largely. Fragments of connective tissue taken from an embryo, or from young adult and old animals and placed in normal adult chicken plasma grew at different rates. The velocity of the growth always varied in inverse ratio to the age of the animal from which the tissue had been extirpated. The various tissues of one animal, or the different parts of one tissue, are not necessarily identical as regards their dynamic condition. In one case, for instance, a small clot of blood in the subcutaneous connective tissue of an adult chicken had become encysted by connective tissue. A few fragments of the cyst wall were cultivated by the ordinary method. After twenty-four hours these fragments of connective tissue were surrounded by a large and dense area of small round and fusiform cells. During the same period of time normal connective tissue from the same region of the body produced only a few large and elongated connective tissue cells, which constituted a network. Two photographs (figures 14 and 15) show the differences in the morphology of slowly and actively growing cells and in the extent of growth after forty-eight hours. The dynamic condition of the connective tissue living

in vitro varied according to the manner in which the cultures were prepared and kept. The greatest activity was observed in the old strain of cells which had been undergoing daily passages for some time past. A comparative study of the proliferation of the cells and of the connective tissue cells of an eight day old chick embryo showed that the quantity of tissue produced in forty-eight hours by the strain adapted to life *in vitro* could be twenty times more abundant than that produced by fresh embryonic tissue. When the tissues were allowed to remain in the medium for two, three, or four days without change, the rate of proliferation decreased. When a fragment of tissue was kept in its medium at a low temperature for four, five, or six days, the rate of growth and the morphological appearance of the cells were modified (figure 16). The cells became larger and their cytoplasm was darker and more abundant. The rate of proliferation had diminished and even after a few repeated passages the growth of the tissue was still slow.

An attempt was then made to determine whether the dynamic condition of the cells was a fixed character that persisted for a long time, or whether it was merely a temporary effect, produced entirely by the medium. Fragments of tissue or strains of cells of different dynamic conditions were placed in identical media, composed either of normal adult chicken plasma or of plasma containing more or less tissue juice. The rate of growth of these tissues which were originally in different conditions of activity was at first very unequal; but after a few passages it became uniform. The strains of cells which had been artificially activated through daily passages, and which in forty-eight hours produced a ring of new tissue of 153.0 or 183.6 microns in width, were, after a few passages, producing a ring of 76.5 or 91.8 microns. Again, the fragments of tissue which originally surrounded themselves in forty-eight hours with a ring of 15.3 or 30.6 microns produced after a few passages a ring of about 91.8 microns. The influence of the medium on the rate of growth was not immediate. For a few days the rate of proliferation depended in large measure upon the previous dynamic condition of the cells; then their activity increased or diminished according to the medium and finally became constant. For instance, a fragment of tissue composed of a strain of cells

which had undergone more than 150 passages, and the growth of which had completely stopped, after having been left for several days in normal chicken plasma, was cultivated in a mixture composed of two parts of plasma and one part of embryonic juice. After twenty-four hours a few cells were seen in the culture medium around the original fragment. After three days the fragment was surrounded by a thin ring, the width of which was 45.9 microns. The fragment was then washed and placed in a medium of identical composition. Two days afterwards the ring of new tissue which had formed was 76.5 microns. After one more passage the rate of growth was entirely normal. The tissues which grew actively and which were placed in the plasma of adult or old animals continued for a few days to develop rapidly. Later the rate of growth diminished progressively. Therefore it seems that the dynamic condition of the cells results from certain substances supplied directly or indirectly by their medium. The activity manifested by the cells for a few days after their medium has been changed may be partly due to the reserves which they have previously accumulated. The action of the medium on the cells is not immediate, but necessarily occurs sooner or later. Hence the conclusion that the dynamic condition of a strain of cells is determined by the medium either immediately or after a short time; or in other words, it is a function of the medium in which it is cultivated.

THE INFLUENCE OF THE CELLS ON THE MEDIUM.

It might be assumed that cells which were actively developing in a medium markedly modified the latter, and rendered it progressively unsuited to their life. Normal death of cultivated tissues was considered as possibly brought about by the exhaustion of the nutritive substances contained in the medium and by the accumulation therein of certain catabolic substances. Some modifications of the culture medium were observed to occur when connective tissue was made to grow actively in Gabritschewski boxes or in test-tubes. The plasmatic jelly around the groups of cells which were rapidly proliferating became thinner, and serum was exuded. The atmosphere of the box became rarefied. When, in cases where tissues were

cultivated in large tubes, the velocity of growth was great, the fibrin which is the framework of the film covering the glass wall soon disappeared and the culture medium appeared to liquefy. The modifications of the atmosphere of the boxes and of the culture medium were merely the manifestations of more profound physico-chemical changes, which will be studied later in greater detail. The only thing which was studied in the present connection was the action on a strain of cells of an extract taken from a culture medium in which normal embryonic tissue had been actively developing. The experiments consisted in adding the fluids from an old culture to the plasma in which the connective tissue cells were being cultivated. The fluids were obtained in the following manner: Normal embryonic tissue was cut into very small pieces, suspended in Ringer solution, and mixed in test-tubes with normal chicken plasma. In some experiments the plasma was allowed to coagulate at the bottom of the tubes; in others, the plasma was made to coagulate in a thin film on the glass wall, whereupon the growth of the tissue became much more rapid. Some of the tubes were placed in cold storage, and the others in the incubator. In the former no growth occurred, while in the latter the tissue grew more or less actively, according to the manner in which the culture had been prepared. After a few days the contents of the tubes were removed. The clot was cut into small pieces and centrifuged and the supernatant fluid as well as the fluid exuded during the growth of the tissue was used for the experiments, one or two parts of the fluid being mixed with one part of plasma. Extracts of cultures were used which had been kept in the incubator in active life or in the refrigerator in latent life. In these media were cultivated strains of connective tissue cells adapted to life *in vitro*. After forty-eight hours the width of the new tissue was measured with a micrometer. The results varied according to whether the cultures that supplied the extract had been kept in the incubator for a short or a long time.

When the embryonic tissues had been growing for one or two days only, the fluid extracted from their medium activated the growth of connective tissue cells. This activating power exceeded that of the fluid extracted from the cultures preserved in cold

storage. Therefore it appeared that the embryonic tissues living actively for a short time had modified the culture medium and rendered it more suitable to the growth of connective tissue cells.

When the cultures of embryonic tissues were allowed to grow more extensively or for a longer time, the properties of their extracts became modified. In one form of experiment the plasma and the suspension of embryonic tissue were placed in tubes and allowed to remain in the incubator for four or five days; in a second, the plasma was distributed over the wall of the tube in a thin layer, the fragments of embryonic tissue being embedded therein at more or less regular intervals. In the latter case the growth was very rapid and in two days the plasma jelly had almost completely liquefied, while the wall of the tube had become covered with continuous and thin layers of living cells. The fluids extracted from the cultures which had been living actively acquired the property of retarding the growth of connective tissue. In a few experiments these fluids caused the proliferation of the cells to cease almost entirely. The experiments show that embryonic tissue which has been actively growing in a culture medium renders it unsuitable for the life of cells.

The results of the experiments lead one to assume that in a culture of tissue progressive diminution of the tissue's rate of growth and its ultimate death are probably brought about by similar modifications of the medium. It was this supposition which enabled me to prolong indefinitely the life of connective tissue *in vitro*. It was found that by washing the tissues of a culture in Ringer solution and placing them in a new medium the rate of their growth did not diminish and death failed to occur. This result could be obtained in several different ways. After a few days of growth in the medium the fragments of tissue were washed in Ringer solution or placed in a large quantity of fluid plasma, where it was thought that the substances which stopped the growth could easily diffuse. When the tissues were transferred to a new medium every twenty-four or forty-eight hours it was unnecessary to wash them; but when they had been allowed to grow for four or five days in a medium the washing in Ringer solution favored the rapidity of their growth. The influence of washing on the growth of tissue was

studied particularly in connection with a strain of cells which had undergone more than 100 passages. Each fragment was divided into two parts, A and B. Part A was washed for thirty seconds or one minute, while part B was placed directly in the new medium. When the tissues were cultivated in a mixture of plasma and embryonic juice and transferred to a new medium every twenty-four or forty-eight hours, the difference in size between parts A and B was very slight and often did not exist at all. However, the new tissue was generally less abundant and less dense in the culture which had not been washed (figure 17, *a* and *b*). The differences were more marked when the tissues were transferred several times without being washed. When the tissues were cultivated in adult chicken plasma without the addition of embryonic juice the difference in growth produced by the suppression of the washing was much more apparent. In this case the tissues which had not been washed often died after a few passages.

The experiments indicate that the growing tissues modify their medium; but the nature of the modifications has not yet been determined.

CONCLUSIONS.

When connective tissue cells have been cultivated for a certain length of time in a medium which has been repeatedly changed, a definite relation arises between the rate of growth of the cells and the composition of the medium. It is possible, by adding to the culture medium a given quantity of certain substances, such as embryonic juices, to foresee the extent to which a fragment of tissue composed of a given strain of cells will increase in a given time. The rate of growth of a strain of cells can be accelerated or retarded by the addition to the medium of activating or retarding substances.

The dynamic condition of a strain of connective tissue cells, which have been living in a given medium for some time, is not a definitely acquired characteristic, but a temporary state, and is the product or function of the medium in which the cells are living, and is readily modified merely by altering the composition of the medium.

A knowledge of the characteristics of the growth of connective tissue described has led to a new result,—the indefinite proliferation of a strain of connective tissue cells outside of the organism. The

strain of connective tissue originally obtained from a fragment of chick embryo heart, which had been pulsating *in vitro* for 104 days, was still actively alive after sixteen months of independent life, and more than 190 passages. The rate of proliferation of the connective tissue sixteen months old equalled and even exceeded that of fresh connective tissue taken from an eight day old embryo. It appears, therefore, that time has no effect on the tissues isolated from the organism and preserved by means of the technique described above. During the sixteenth month of life *in vitro* the cells increased rapidly in number and were able in a short time to produce a large quantity of new tissue. This fact, therefore, definitely demonstrates that the tissues were not in a state of survival, as was the case in certain earlier experiments, but in a condition of real life, since the cells of which they were composed, like microörganisms, multiplied indefinitely in the culture medium.

EXPLANATION OF PLATES.

PLATE 10.

FIG. 1. Culture 5,200-1. March 10, 1913, fourteenth passage of connective tissue. Medium, adult chicken plasma. Photograph taken on March 12, 1913.

FIG. 2. Culture 5,200-2. March 10, 1913, fourteenth passage of connective tissue. Medium, two parts adult chicken plasma, one part embryonic juice. Photograph taken on March 12, 1913.

PLATE 11.

FIG. 3. Culture 5,166. March 10, 1913, seventy-two hours after the 151st passage. A₁, cultivated in normal plasma; B₁, cultivated in two parts normal plasma and one part embryonic juice.

FIG. 4. Same tissues. March 12, 1913, forty-eight hours after the 152d passage. A₂, cultivated in normal plasma; B₂, cultivated in two parts normal plasma, one part embryonic juice.

FIG. 5. Same tissues. March 14, 1913, forty-eight hours after the 153d passage. A₃, cultivated in two parts plasma and one part embryonic juice; B₃, cultivated in normal chicken plasma.

FIG. 6. Same tissues. March 16, 1913, forty-eight hours after the 154th passage. A₄, cultivated in two parts plasma and one part embryonic juice; B₄, cultivated in normal chicken plasma.

PLATE 12.

FIG. 7. Culture 3,579. Photograph taken on January 18, 1913, forty-eight hours after the 130th passage.

FIG. 8. Same tissue. Photograph taken on January 20, 1913, forty-eight hours after the 131st passage.

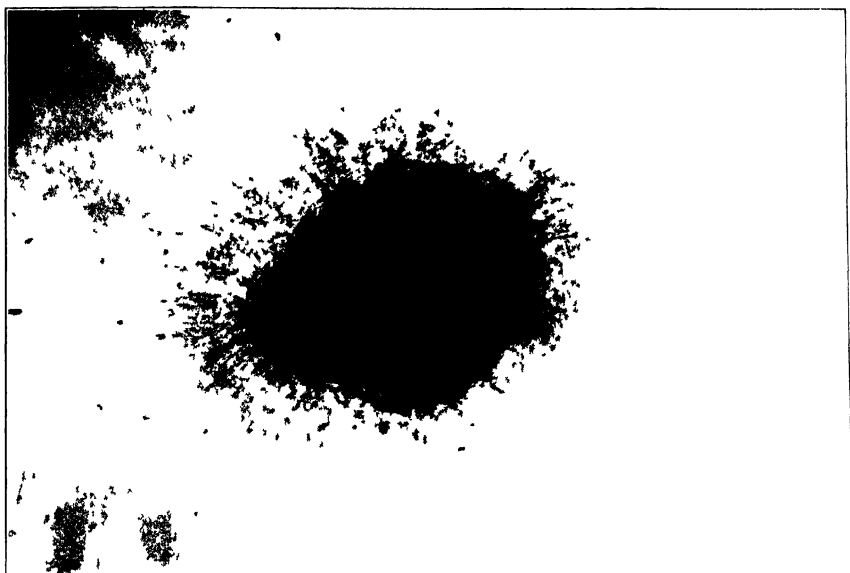


FIG. 1

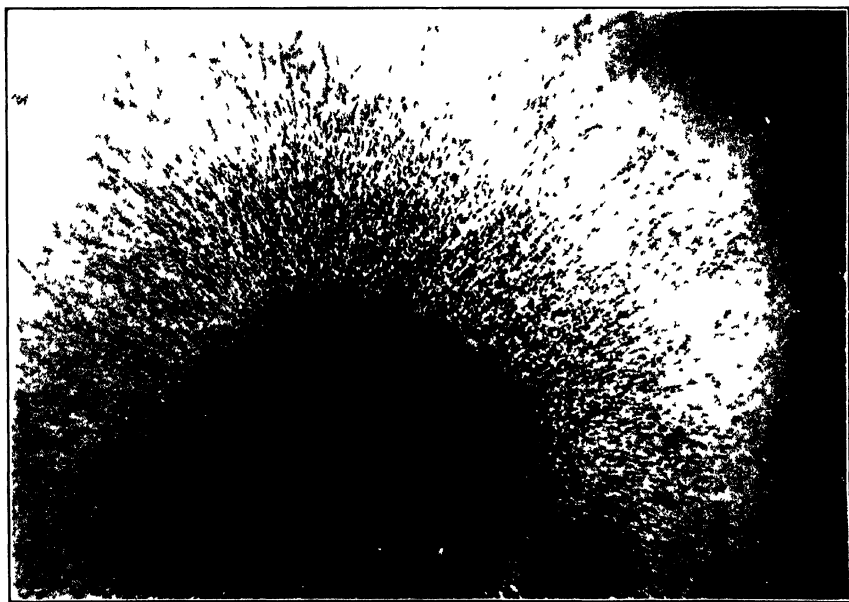


FIG. 2

(Control Mechanism of Growth of Connective Tissue)

Fig 3

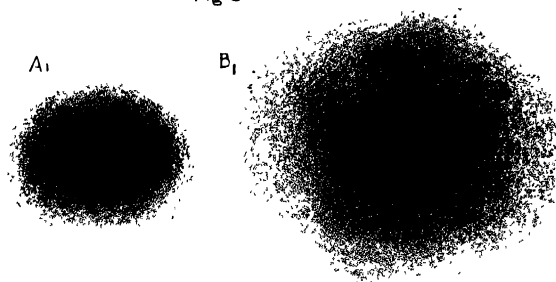


Fig 4

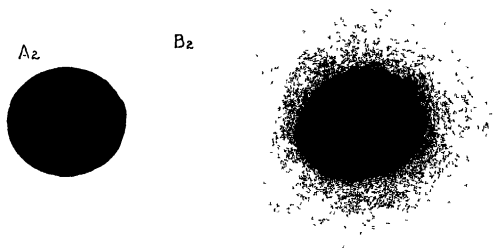


Fig 5

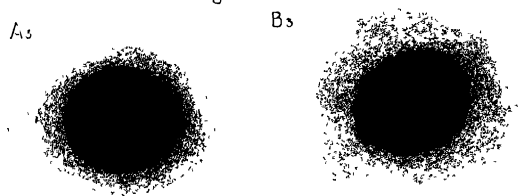
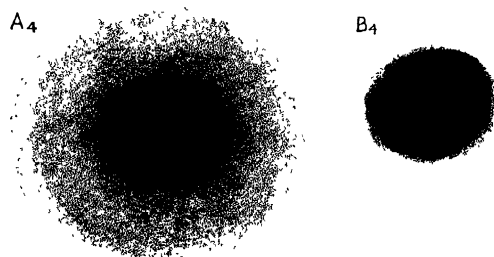


Fig 6



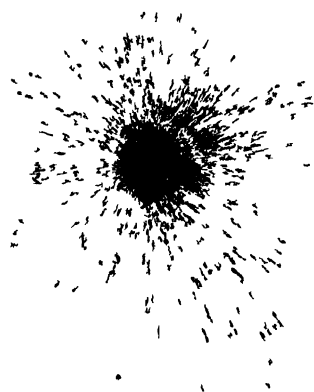


Fig. 7

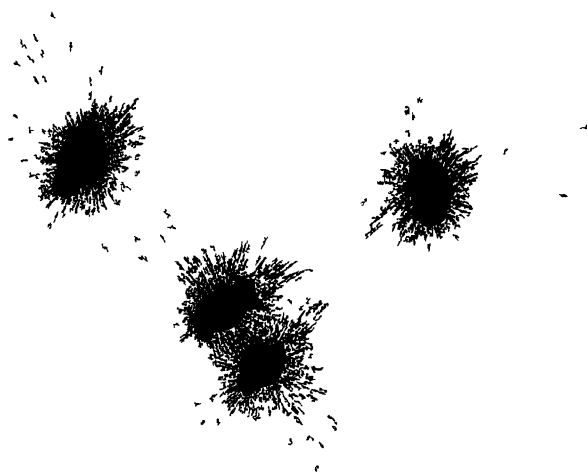


Fig. 8

(Cell Material from Growth of Connective Tissue)

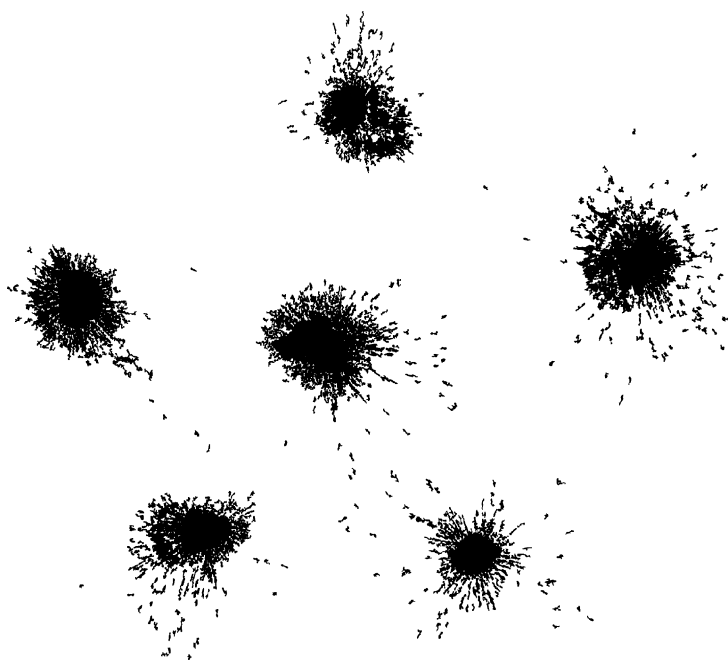
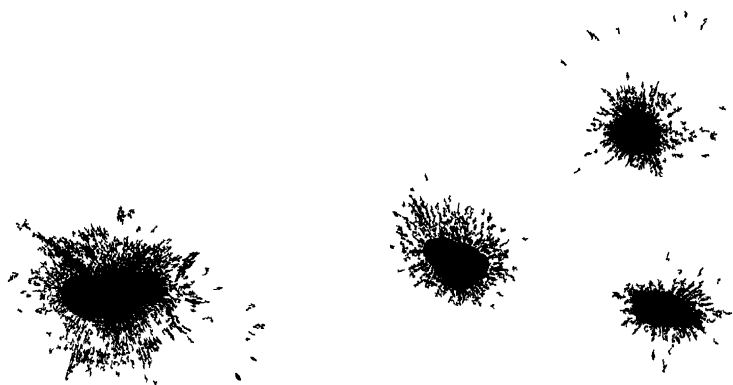


FIG. 11

(Circular Medium of Growth of Connective Tissue)

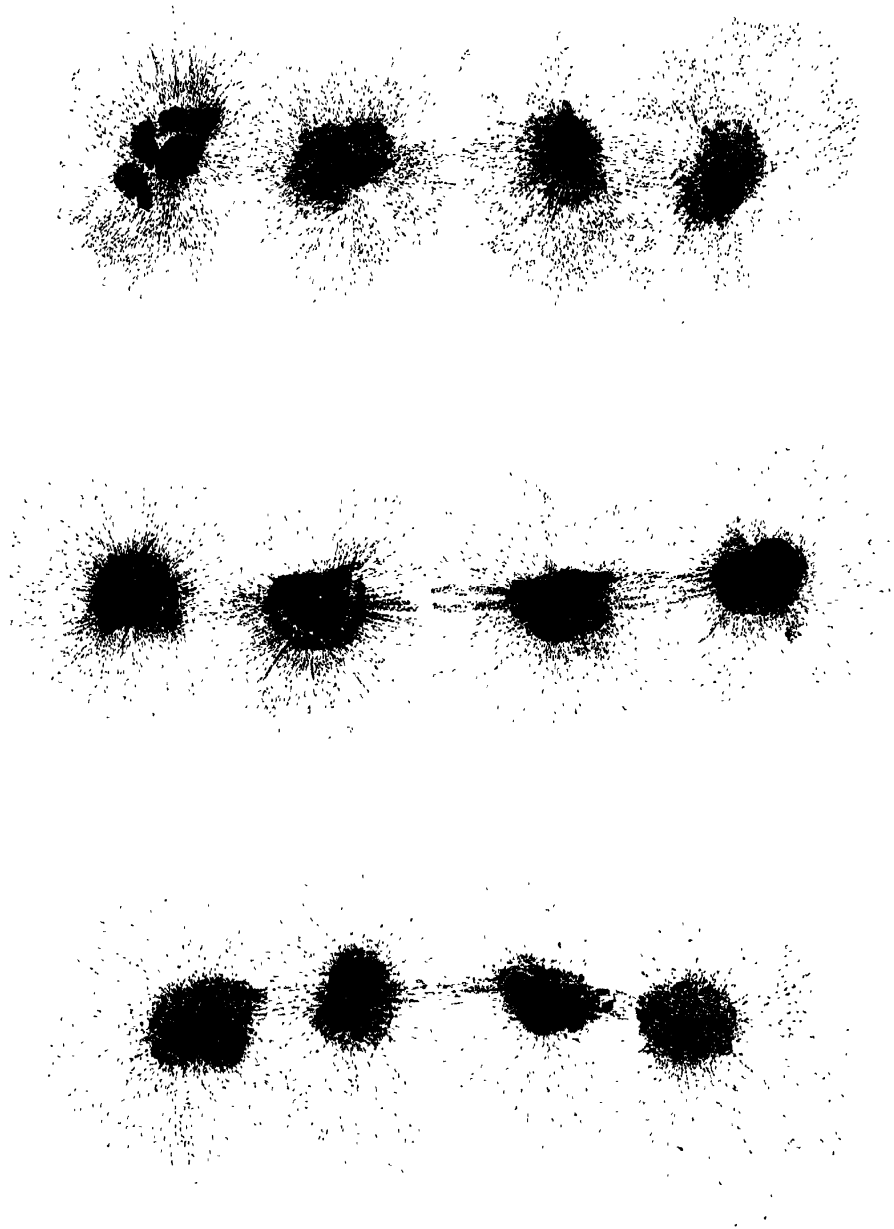


FIG. 12.

(Carel) Mechanism of Growth of Connective Tissue



FIG. 13

(Carrel—Mechanism of Growth of Connective Tissue.)



FIG. 14

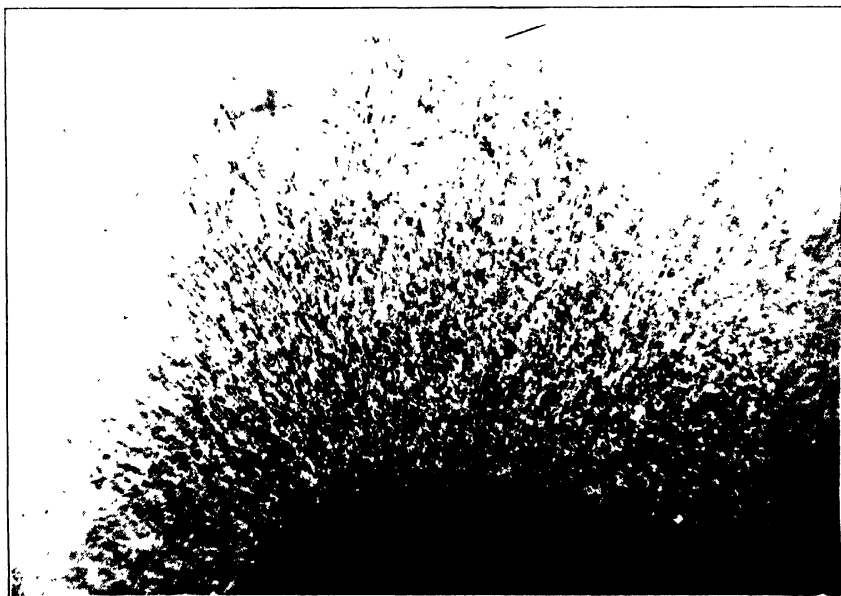


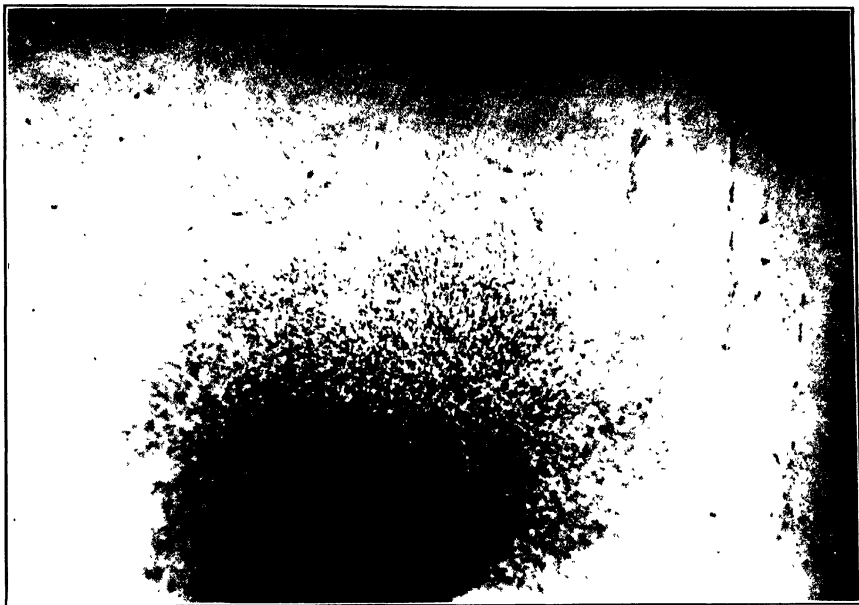
FIG. 15

(Carrel: Mechanism of Growth of Connective Tissue)



FIG. 16

(Control Mechanism of Growth of Connective Tissue)



a



b

FIG. 17.

(Carrel Mechanism of Growth of Connective Tissue.)

PLATE 13.

FIG. 9. Culture 3,575. Photograph taken on January 17, 1913, seventy-two hours after the 87th passage.

FIG. 10. Same tissue. Photograph taken on January 20, 1913, forty-eight hours after the 89th passage.

FIG. 11. Same tissue. Photograph taken on January 22, 1913, forty-eight hours after the 90th passage.

PLATE 14.

FIG. 12. Same tissue. Photograph taken on January 27, 1913, seventy-two hours after the 92d passage.

PLATE 15.

FIG. 13. Connective tissue cultivated in a test-tube after fourteen months of life *in vitro*. The photograph shows a growth of forty-eight hours.

PLATE 16.

FIG. 14. Culture of connective tissue of adult chicken, after forty-eight hours.

FIG. 15. Culture 5,200-2. Fourteenth passage of connective tissue, growing very actively.

PLATE 17.

FIG. 16. Culture of connective tissue after seven days in an incubator at a temperature of 35° C.

PLATE 18.

FIG. 17. Culture 5,192. Photograph taken on March 13, 1913, seventy-two hours after the 155th passage. *a* had been washed for one month in Ringer solution. *b* had not been washed.

REGENERATION OF AXIS CYLINDERS IN VITRO.

SECOND COMMUNICATION.*

By RAGNVALD INGEBRIGTSEN, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research,
New York.)

PLATES 27 TO 31.

In a previous article¹ I described certain observations on cultures made from pieces of the central nervous system of young mammals, cultivated in coagulated plasma. Most of the observations were made from living (unstained) material. Further study of the cultures, carried on with different methods of fixation and staining, has confirmed the observations made on unstained preparations; namely, that nerve fibers grow out from pieces of the cerebellum and spinal ganglia of young mammals, when cultivated in plasma. Certain other phenomena of growth have also been revealed. This paper, however, will be limited to a description of the outgrowth of nerve fibers and their appearance in stained specimens.

As young dogs were not available, young cats and guinea pigs were employed in most of the experiments. The cultures were chiefly made from pieces of cortex of the cerebellum and spinal ganglia.

Held's pyridin method was found to be the only practicable one for fixing the cultures. It causes no precipitation in the plasma, and the subsequent staining by Cajal's silver nitrate method, when successful, has given good results. In good preparations the fragment of tissue stains dark brown or black and the surrounding plasma a light reddish brown. The black or dark brown nerve fibers which have grown out are easily discernible against this background.

Cerebellum.—The network of dark brown or black nerve fibers

* Received for publication, June 17, 1913.

¹ Ingebrigtsen, R., *Jour. Exper. Med.*, 1913, xvii, 182.

contained in the tissue can be observed in the thinner part of the transplanted fragment, especially along the border lines adjoining the plasma. In some preparations the new fibers in the plasma can be determined as a direct outgrowth or continuation of fibers contained in the tissue itself (figure 1). In other preparations this direct connection is not visible, either because the original fragment fails to reveal individual fibers or because it is surrounded by other newly grown tissues (connective tissue, glia), which cover the nerve fiber at the point where it emerges from the brain and enters the plasma (figure 2).

From this point the nerve fiber often proceeds by means of a terminal bulb, which in the living culture possesses ameboid movements. In stained preparations the terminal bulb usually takes on a somewhat lighter shade of brown than the fiber itself and shows an even, slightly granular structure. It is ovoid or globular in shape with a diameter of two to three microns. No capsule has ever been seen surrounding it. This end bulb is not, however, invariably present; sometimes the fiber proceeds with a point, and in one preparation the swelling at the end of the fiber is probably produced by a minute loop formed by its point (figure 3).

Throughout their course the fibers are either evenly cylindrical (figure 2) or provided with varicosities, which have much the same appearance as the terminal bulb (figures 1 and 4).

Spinal Ganglia.—While the cultures of cerebellum in this series of experiments with stained specimens have shown only a scant number containing newly grown nerve fibers, the cultures of spinal ganglia give better results both as regards the number of successful cultures and the number of nerve fibers growing out in each culture.

The technique employed for the preparation was the same in both cases: the pieces of cerebellum, as well as of the spinal ganglia, were taken from the living etherized animal, put directly into Ringer solution, where they were cut, and from there transferred to the plasma.

The success of the operation depends upon the rapidity with which it is carried on, at least in the case of the cultures of the cerebellum, twelve to fifteen seconds being the longest lapse of time permissible between the removal of the tissue from the animal and

its inoculation into the plasma. In the case of the spinal ganglia the interruption of the circulation and the transplantation to the new medium is more easily withstood without injury.

Most of the cultures of spinal ganglia, as well as those of cerebellum, were fixed and stained after two or three days' incubation, in order to be sure that degenerative changes of the newly grown fibers had not occurred; as a rule, these changes begin to appear on the fifth and following days at an incubation of 39° to 40° C. On the other hand, the growth of the nerve fibers is most rapid during the first two days, and on the second day, in particular, they attain, if not their full size, at least almost their entire length.

In the cultures of spinal ganglia, as well as in those of cerebellum, the nerve fibers in the plasma in several preparations have grown out as a direct continuation of the fibers in the original piece, and the outgrowth of new fibers is especially abundant in places where parallel and transversely cut nerve fibers radiate out against the plasma. The nerve fibers are also seen to grow out from masses of ganglia cells, situated at the edges of the fragment (figure 5).

The nerve fibers growing out from the spinal ganglia generally reach a greater length than those from the cerebellum, many of them attaining 500 to 600 microns after forty-eight hours. The different fibers vary somewhat in thickness, and the heaviest ones branch several times. They are mostly cylindrical and absolutely even throughout their course, but they bend and curve in various directions, and sometimes spirally. Most of them end in a point. In one preparation most of them end in a bulb, and in this case the fibers are at intervals provided with varicosities, spindle-shaped, ovoid, or globular in form. These have a slightly granular structure and the centers stain somewhat darker than the peripheral parts.

In none of the stained cultures, whether taken from the cerebellum or the spinal ganglia, have I observed a true anastomosis. In the previous paper I described the existence of anastomoses between some of the fibers in the living cultures. This phenomenon has not been confirmed in the stained specimen, and its interpretation will therefore require further study. It may be merely a coalescence between two fibers situated closely together.

The nerve fibers from the cerebellum, as well as those from the



FIG. 1.

(Ingebrigtsen Regeneration of Axis Cylinders in Vitro)

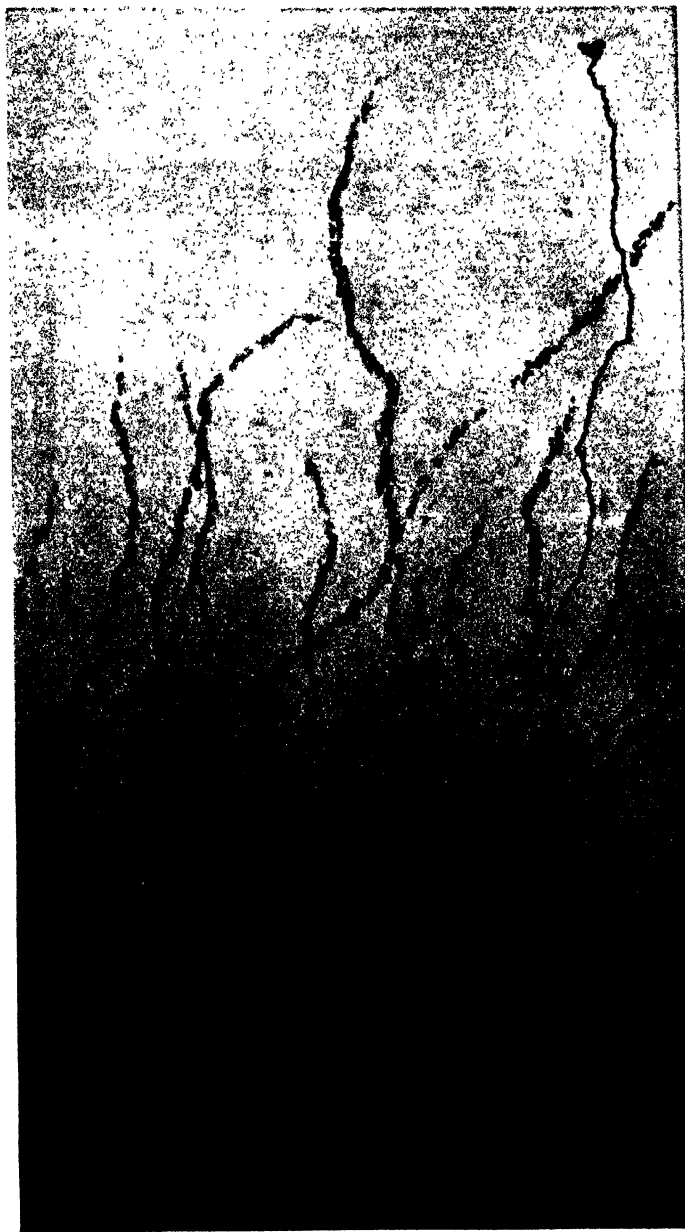


FIG. 2.

(Ingebrigtson: Regeneration of Axis Cylinders in Vitro.)



FIG. 3.

(Ingebrigtsen: Regeneration of Axis Cylinders in Vitro.)

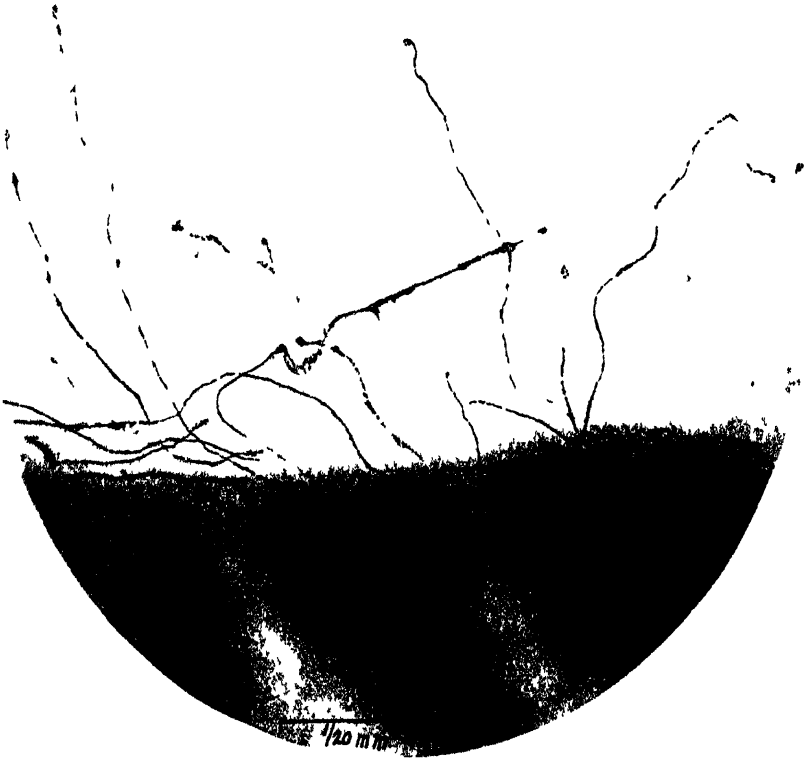


FIG. 4

(Ingebrigtsen: Reactions of Axis Cylinders in Vitro.)

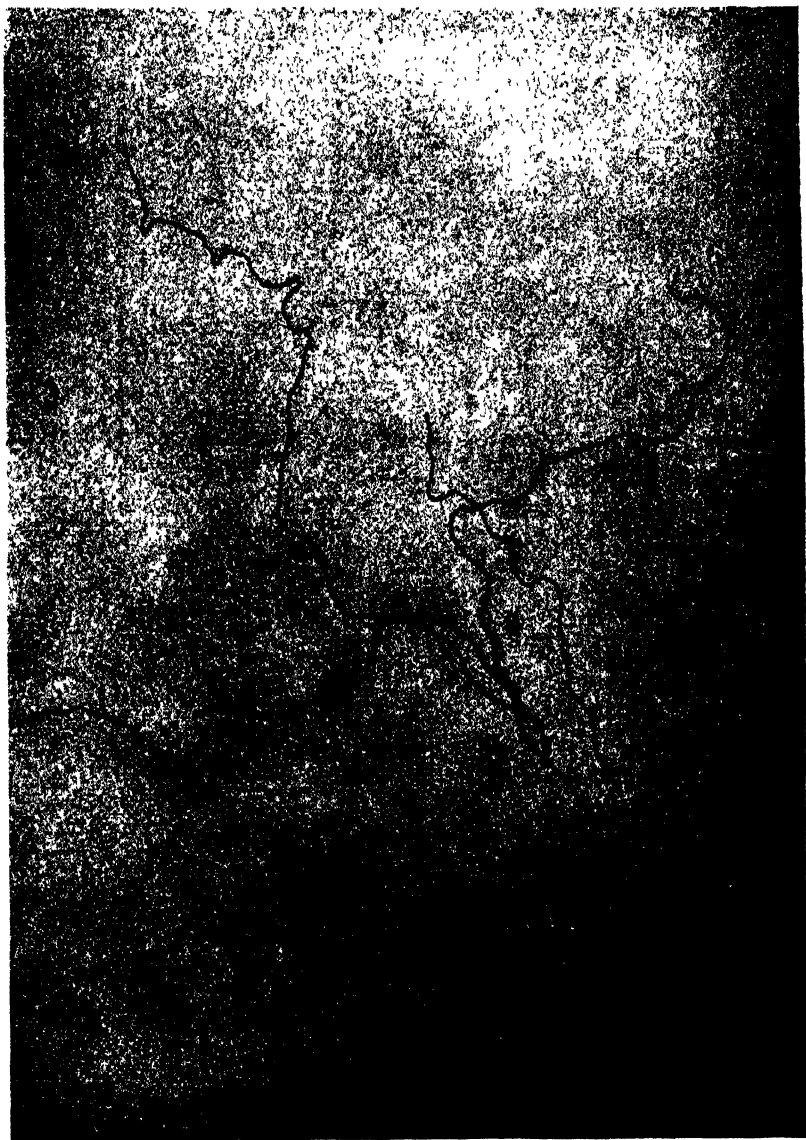


FIG. 5

(Ingebrigtsen—Regeneration of Axis Cylinders in Vitro)

spinal ganglia, emanate freely into the plasma without the support or attachment of any other structure. They are not accompanied by other tissue; sometimes they are surrounded by connective tissue cells or glia cells, but their growth begins earlier and does not depend upon the presence of these structures. In fact, cultures with rich growths of connective and glia tissues rarely contain newly grown nerve fibers.

SUMMARY.

It has been shown for the first time that nerve fibers grow out from pieces of cerebellum of young cats and guinea pigs, when cultivated in coagulated plasma.

The same phenomenon has been observed in cultures of spinal ganglia.

The nerve fibers do not anastomose and they extend into the plasma unaccompanied by structures of any kind.

EXPLANATION OF PLATES.

Figures 1, 2, 4, and 5 are camera lucida drawings focused on different planes of the plasmatic medium, in order to bring out the nerve fibers throughout their entire length.

PLATE 27.

FIG. 1. Two day old culture of cerebellum of a guinea pig eight days old.

PLATE 28.

FIG. 2. Two day old culture of cerebellum of a guinea pig eight days old. A single nerve fiber is seen surrounded by other structures, probably neuroglia.

PLATE 29.

FIG. 3. Photographs of a two day old culture of cerebellum of a cat six days old. The photographs are taken at two different magnifications, 1,300 and 1,900 diameters respectively.

PLATE 30.

FIG. 4. Two day old culture of cerebellum of a guinea pig eight days old.

PLATE 31.

FIG. 5. Two day old culture of spinal ganglia of a guinea pig six days old.

WATER-IMMISCIBLE ORGANIC LIQUIDS AS CENTRAL CONDUCTORS IN GALVANIC CELLS.

By REINHARD BEUTNER.

(From the Laboratories of The Rockefeller Institute for Medical Research, New York.)

A year ago I had the privilege of presenting a paper before this Society¹ on electromotive forces generated by systems containing immiscible electrolytic conductors. I also mentioned in that paper some investigations relating to biological applications of the electrochemical researches along this line carried out by Dr. J. Loeb and the writer, in which it had been found that the skin of plants behaves like an electrode reversible with respect to various positive ions.

As I pointed out, a phenomenon of this kind was not yet known in electrochemistry, though certain investigations of Nernst and of Haber (which were there discussed at length) had shown that a phase junction between immiscible electrolytes acts like the surface of a metallic electrode. This did not explain how such an effect was possible.²

More recent investigations which the writer has carried out have proved that with a number of water-immiscible organic substances similar effects can be produced as with the skin of plants. This has led to further investigations into the electromotive properties of water-immiscible organic substances, and the surprising result has been reached that cells may be built up from such organic compounds and aqueous solutions showing an electromotive force which nearly equals in magnitude the E. M. F. of galvanic cells with metallic electrodes. An explanation of these phenomena has been

¹ Beutner, Trans. Am. Electrochemical Society, 21, 219 (1912).

² The explanation given, that "a chemical reaction takes place between the aqueous solution and a water-insoluble compound of the membrane causing an exchange," was hardly more than a very indefinite hypothesis, and it seems rather accidental that it is right in some degree, as we shall see later.

found possible on the basis of the well-known theories of chemical thermodynamics. The quantitative mathematical applications involved, however, many difficult questions.

I. SALICYLIC ALDEHYDE AS ELECTRODE.

Nernst's law for potential differences at the junction of immiscible phases holds as follows:

$$e = \frac{RT}{nF} \ln \frac{C_1}{C_2} + \text{const.}$$

as was proved in the simplest manner possible in my previous communication. The meanings of R , T , n and F are as usual, while C_1 and C_2 are the respective concentrations of the same ion in the two immiscible phases. If one of these phases is an aqueous solution of various salts, and the second a homogeneous mixture of a great number of water-insoluble salts, we can easily understand from this formula that any of these various ions contained in the second phase influences the potential difference if it is contained in the aqueous solution in a concentration subject to arbitrary change.

The fact, however, is that chemically-pure water-immiscible substances exhibit a behavior of this kind. The best example of a substance of this character that I have been able to find so far is salicylic aldehyde, which behaves like an electrode for various cations.

In the following tables the sign of the E. M. F. is marked as + if the right side of the cell is positive.³

TABLE I.

Hg Hg ₂ Cl ₂ . $\frac{m}{10}$ K Cl		sat. salicylic acid in salicylic aldehyde	
aqueous solution variable composition		$\frac{m}{10}$ K Cl . Hg ₂ Cl ₂ Hg	

³ These e. m. f.'s were measured by means of Dolezalek's electrometer. This instrument, in which the deflections are proportional to the e. m. f. applied, was standardized with the Clark standard cell. The experimental arrangement used to build up the "cell" systems was very simple, a U tube containing the salicylic aldehyde.

E. M. F., using variable aqueous solutions, as follows:

Concentration (Mol. fractions per liter)	KCl E. M. F. Millivolts	NaCl E. M. F. Millivolts	Na ₂ SO ₄ E. M. F. Millivolts	CaCl ₂ E. M. F. Millivolts	NH ₄ Cl E. M. F. Millivolts
5 m/2	— 39	..	..	..	..
m/2	— 21	..	..	..	..
m/10	0	+ 16	+ 16	+ 37	0
m/50	+ 24	+ 38	+ 32	+ 54	+ 24
m/250	+ 54	+ 64	+ 44	+ 82	+ 50
m/1250	+ 89	+ 90	+ 73	+ 100	+ 82
m/6250	+ 130	+ 124	..	+ 124	..

Concentration (Mol. fractions per liter)	CuSO ₄ E. M. F. Millivolts	AlCl ₃ E. M. F. Millivolts	Na Acetate E. M. F. Millivolts	H ₂ SO ₄ E. M. F. Millivolts	HgCl ₂ E. M. F. Millivolts
m/10	+ 14	+ 7	+ 64	— 10	+ 120
m/50	+ 24	+ 32	+ 68	+ 20	..
m/250	+ 35	+ 52	+ 82	+ 56	+ 159
m/1250	+ 57	+ 69	+ 113	+ 83	(at m/100)

All these measurements, which were performed at room temperature, prove that the change of the E. M. F. with concentration occurs in the same direction with all salts. The point where this change takes place must be the phase junction between the salicylic aldehyde and the solution, because the change of the "liquid potential" located at the junction of the calomel electrode and the solution is too small to account for the total change observed. In the first series of measurements (with KCl solutions) there is no liquid potential difference at all; since the migration velocities of the K ions and the Cl ions are very nearly equal, and the factor $U_K - V_{Cl}' / U_K + V_{Cl}'$ is essential for liquid potentials, the liquid potential is practically zero. The change is in this case due to the potential difference at the phase junction exclusively.

The measurements with KCl are, therefore, most suitable for studying these new potential differences. The magnitude of the change increases as the dilution decreases and approaches the value of 41 millivolts at very low concentrations. This is the value which we should expect according to Nernst's formula if the aldehyde acts like a perfect K electrode.

$$\frac{RT}{nF} \ln 5 = 58 \log 5 = 40 \text{ millivolts}$$

This fact is very important for the theoretical explanation, as we shall see later.

The same change of the potential difference is observed with all other salts. However, the "liquid potential" interferes in these cases to some extent. The measurements are reversible as a rule,⁴ except in some cases, *e. g.*, AlCl_3 , where irreversible chemical processes interfere.

The summary of these observations is that salicylic aldehyde acts like an electrode reversible for various cations. Equimolecular solutions of different salts, however, do not always produce the same potential difference. This is very clearly seen from the measurements with HgCl_2 . The system:

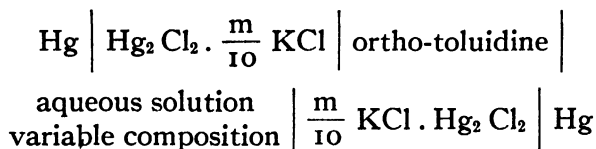
—Calomel electrode | salic. ald. | $n/10 \text{ HgCl}_2$ | calom. elec. + has a considerable E. M. F. (0.12 volt), which is also perfectly reversible, as the following figures show:

Time in Minutes.	Solution.	E.M.F. Millivolts.
0	$m/10 \text{ HgCl}_2$	+ 120
8	$m/10 \text{ KCl}$	+ 19
15	$m/10 \text{ KCl}$	+ 0

The last value for $m/10 \text{ KCl}$ is identical with that in the above table.

II. TOLUIDINE AS ELECTRODE.

Table II contains measurements of the E. M. F. of the system:



In this case the organic liquid used (ortho-toluidine) behaves like

TABLE II.

Concentration (Mol. fractions per liter)	KCl E.M.F. Millivolts	NaCl E.M.F. Millivolts	CaCl_2 E.M.F. Millivolts	AlCl_3^* E.M.F. Millivolts	NaOH E.M.F. Millivolts
$m/10$	0	0	0	—28	—19
$m/50$	—19	—22	—25	—33	—42
$m/250$	—49	—55	—51	—66	—51
$m/1250$	—72	—87	—85	..	—75
$m/6250$	—94	—109	..	..	..

* These measurements are irreversible.

⁴ This means that in a series of measurements with different concentrations of the same salt the potential difference comes back instantly to its previous value if the same concentration is applied again.

Concentration (Mol. fractions per liter)	KSCN E.M.F. Millivolts	KCN E.M.F. Millivolts	KNO ₃ E.M.F. Millivolts	K ₄ FeCy E.M.F. Millivolts	Cu ₂ SO ₄ E.M.F. Millivolts
m/2	+ 156	+ 57	—	—	—
m/10	+ 129	+ 36	+ 60	— 100	— 122
m/50	+ 100	+ 14	+ 32	— 113	— 134
m/250	+ 64	+ 4	— 3	— 126	— 146
m/1250	+ 26	..	— 31	— 144	— 154
m/6250	— 16	..	— 71	..	..
m/31250	— 62	..	— 103	..	..

Concentration (Mol. fractions per liter)	Na ₂ SO ₄ E.M.F. Millivolts	NaF E.M.F. Millivolts	Na ₂ HPO ₄ E.M.F. Millivolts	Other Salts E.M.F. Millivolts	E.M.F. Millivolts
m/10	— 114	— 100	— 107	m/10 MgSO ₄	— 125
m/100	— 130	— 133	— 125	m/10 NH ₄ Cl	0
m/1000	..	— 137	..	m/10 BaCl ₂	0
m/10 Toluidine hydrochloride — 23. m/50 K ₂ Cr ₂ O ₇ + 38					

an electrode reversible with respect to anions. The E. M. F. becomes more negative with decreasing concentration.

These figures show that toluidine has properties with respect to the electromotive forces which are equal and opposite to those of salicylic aldehyde. The potential difference changes in the opposite direction to the concentration, and we may call the potential difference, therefore, reversible for anions. As we should expect, the chemical nature of the anion is most significant for the potential difference in this case. NaCl, KCl and CaCl₂ give in equi-molecular concentrations about the same potential difference, evidently because they possess the same anion, while with salicylic aldehyde KCl and CaCl₂ gave markedly different potential differences as a result of the reversibility for cations in this case. Salts with different anions, however, like NaF and KSCN give largely-differing potential differences with toluidine, the first a considerably negative value, the second a high positive value. If we combine these two potential differences in one system, as follows:

Cal. Electr. | m/10 NaF Sol. | toluid. | m/10 KSCN | Cal. Electr. +
an E. M. F. of 0.23 volt is produced.

Other anions which give a positive potential difference at the same concentration are NO₃' and CN'', while SO₄'', Fe(Cn)₆''', C₂H₃O' and POH'' give a "negative" value. It may be remem-

bered that with all these figures the Cl' is taken as arbitrary zero, as the calomel electrode used was filled with $m/10$ KCl solution, and this solution constantly remained in contact with the toluidine on the one side.

The rule that the chemical nature of the anion determines the potential difference at the toluidine is not without exceptions. Thus, for instance, AlCl_3 in a $1/10$ molecular concentration exhibits a negative value distinctly different from that of KCl, NaCl, CaCl_2 ; it is found, however, that the potential difference undergoes irreversible changes with the concentration, and there are, therefore, most likely irreversible chemical processes which cause this exceptional behavior.

The figures show very clearly that the direction of the change of the potential difference is the same with all salts; it is observed, however, that with salts which even at comparatively high concentrations exhibit a "negative difference," as fluorides, sulphates, etc., the potential difference becomes only slightly more negative with diminishing concentration. The reason is evidently that in these cases the presence of even the slightest trace of salt with a higher positive potential difference interferes; if, for instance, the distilled water which is used to dilute the solution of the sulphate contains the slightest trace of a chloride, the potential difference can apparently not become more negative than corresponds to this very small chloride concentration. Considering that the chloride potential difference is much more positive, we understand why a small Cl' concentration interferes with the SO_4 potential difference.

The influence of the concentration upon the potential difference can for this reason be studied over a large range only with such salts as have a high positive potential difference, like KSCN; these measurements show in a very striking way how very regular and easily reproducible the change of the potential difference is. The change is larger at small concentrations, and approaches at very small concentrations the value of about 40 millivolts for a five-fold dilution. This should be expected from Nernst's formula, if the toluidine acted like a perfect SCN' electrode (or if the SCN' concentration in the toluidine were constant).

III. EXPERIMENTS WITH MIXTURES OF TOLUIDINE AND SALICYLIC ACID WHICH EXPLAIN THE NATURE OF THE E. M. F. DESCRIBED ABOVE.

Toluidine has a marked basic character, while salicylic acid contained in the salicylic aldehyde is a strong acid.⁵ It is evident that the opposite change of the potential difference with the concentration in the two cases has something to do with the basic and with the acid character of the compounds used.

I have performed a large number of experiments with other compounds immiscible with water. Such substances as amylalcohol, benzyl alcohol, phenol, ortho-kresol or Beta-naphthol, which do not possess a marked acid or basic function, exhibit either a very small change of the potential difference (with KCl solutions) or none at all. Bases, such as aniline, methylaniline, naphthylamine, act like toluidine; or if we dissolve a water-insoluble acid like salicylic acid in phenol or kresol, an electromotive effect similar to that of the salicylic aldehyde (which always contains salicylic acid) is produced.⁶

I have also measured the potential difference with benzaldehyde instead of salicylic aldehyde; this aldehyde naturally contains benzoic acid through oxidation with atmospheric oxygen; a similar electromotive effect to that with salicylic aldehyde is therefore observed, but the change of the potential difference with the concentration is much smaller, apparently owing to the weaker acid nature of benzoic acid as compared with that of salicylic aldehyde.

There can be no doubt, therefore, that the degree of the acid or basic properties of the water-immiscible substances is responsible for the potential differences described. The question now arises how that fact can be explained from a theoretical standpoint.

Though probably a great many explanations may be possible, only those now appear satisfactory which are based on purely thermodynamic considerations, and we therefore make Nernst's formula for phase junctions the starting point of our theory.⁷ Such

⁵ Ostwald's "Affinitäts Konstante" has the high value of 0.102 for salicylic acid.

⁶ Beutner, Journal of the Am. Chem. Soc., 35, 344 (1913).

⁷ Page 307.

conceptions as, for instance, ionic permeability,⁸ though still often used by some investigators, are not really based on thermodynamics, and, moreover, are found to be contradictory to observations.

Measurements of the potential difference with mixtures of toluidine and salicylic acid have served to find a satisfactory explanation. Equi-molecular parts of salicylic acid and toluidine were dissolved in ortho-kresol, and the potential differences measured with various salt solutions. No considerable change of the potential differences with the concentration is observed if the electrolyte dissolved in the aqueous solution is a neutral inorganic salt, as the following measurements with KCl solutions prove:

TABLE III.

Concentration of KCl sol.	E. M. F. millivolts
m/10	— 5
m/100	— 3
m/1000	+ 1

This is what we should expect on account of the fact that toluidine and salicylic acid (in the aldehyde) cause inverse effects.

The toluidine-salicylic acid mixture in the kresol contains toluidine cations and salicylic acid anions in a constant concentration. A "reversibility" for cations should therefore be observed if toluidine chloride is the electrolyte of the aqueous solutions, a reversibility for anions, on the other hand, with sodium salicylate. In the first case the ion common to both phases (kresol and water) is the toluidine cation; as the kresol concentration of it is kept constant, Nernst's formula for phase junctions provides a change of the potential difference in the same direction as at a metallic electrode if the aqueous concentration is changed. In the second

⁸ The conception of "selective ionic permeability" was originated by Ostwald in 1890 (*Zeit. phys. Chem.*, 6, 11). Though it was proved to be contradictory to facts by very conclusive experiments of Tammann in 1891 (*Göttinger Nachrichten*, 6, 213), this conception was introduced into electrophysiology by Bernstein and adopted by many authors in that field. Freundlich some time ago introduced the conception that potential differences may be produced by "Ionic Absorption," which was also adopted by some electrophysiological authors; in a more recent publication, however, Freundlich admits, himself, that this principle fails to give a satisfactory explanation of electro-endosmotic experiments (Freundlich and Elissahoff, *Zeit. phys. Chem.*, 79, 385).

case the salicylic acid anion is common to both phases, and the change of the potential difference should therefore take place in the opposite direction.

The following experiments prove that this is really the case, within a certain range of concentrations.

TABLE IV.

$\text{Hg} \left \text{Hg}_2\text{Cl}_2 \cdot \frac{m}{10} \text{KCl} \right $		equimolecular solution of salicylic acid and o-toluidine in o-kresol	$\left \frac{m}{10} \text{KCl} \cdot \text{Hg}_2\text{Cl}_2 \right \text{Hg}$	
aqueous solution			variable composition	
Concentration	Toluidine Chloride Solution	E.M.F. Millivolts		Sodium Salicylate Solution
m/2	— 58	E. M. F. increasing	+ 53	E. M. F. decreasing
m/10	— 27	with decreasing	+ 20	with decreasing
m/50	— 9	concentration	+ 2	concentration

The fact that the change is not quite as large as at metallic electrodes (40 m. v.) can be explained, first, by the solubility of toluidine salicylate in water, and second, by the solubility of toluidine chloride and sodium salicylate in kresol. Only in case these solubilities would be practically zero, which apparently is not the case, could the value of 40 m. v. be expected.

A change of the potential difference under such conditions is observed in the same respective directions if, instead of toluidine chloride in the aqueous solution, aniline chloride or methylaniline chloride is used, or if, instead of sodium salicylate, sodium benzoate or acetate is used:

TABLE V.

$\text{Hg} \left \text{Hg}_2\text{Cl}_2 \cdot \frac{m}{10} \text{KCl} \right $		equimolecular solution of salicylic acid and o-toluidine in o-kresol	$\left \frac{m}{10} \text{KCl} \cdot \text{Hg}_2\text{Cl}_2 \right \text{Hg}$	
aqueous solution			variable composition	

Concentration (Molecular Fractions per liter)	Aniline Chloride Solution E.M.F.(m.v.)	Methylaniline Chloride Solution E.M.F.(m.v.)	Sodium Benzoate Solution E.M.F.(m.v.)	Sodium Acetate Solution E.M.F.(m.v.)
m/2	— 50	— 52	+ 58	+ 41
m/10	— 25	— 36	+ 20	+ 17
m/50	— 6	— 14	+ 4	+ 6

These figures show that a reversibility with respect to the various ions (anions or cations) is also exhibited by the toluidine-salicylic acid mixture. *The "organic" ion of the salt in the aqueous solutions determines the direction of the change*; salts with "organic" cations produce a reversibility with respect to cations, salts with "organic" anions a reversibility with respect to anions.

The explanation which seems to me most likely to account for these phenomena is based upon the assumption that a chemical reaction takes place between the electrolytes of the kresol and the water, and that the products formed by this reaction have in equilibrium a different distribution between the two solvents.

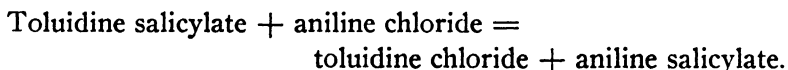
In connection with Nernst's formula this can explain the observation, for instance, with the potential difference:

toluidine salicylate
in o-kresol

|

aniline chloride
in water

as follows: Aniline chloride is soluble to some extent in kresol and toluidine salicylate in water. In both solvents, therefore, in the vicinity of the phase junction the following reaction may take place:



If we assume that the aniline salicylate is more soluble in the kresol than the toluidine chloride, the effect of this exchange will be such that the aniline cations concentrate in the kresol, or, in other words, the concentration of aniline ions in the kresol does not change as the concentration of the aniline ions in the water, but is either constant and independent of the aqueous concentration of aniline cations or changes at least at a smaller rate. The aqueous concentration of aniline cations is due only to the dissociation of aniline chloride, while in the kresol both aniline salicylate and aniline chloride produce such cations. If the kresol concentration of the

aniline salicylate was constant and much larger than the concentration of aniline chloride, the phase junction apparently would exhibit the maximum change of the potential difference (40 millivolts for a 5-fold dilution), because the concentration of the aniline cations in the kresol would thus be constant. Nernst's formula for phase junctions, which in this case can be applied as follows:

$$e = \frac{RT}{F} \log \frac{C \text{ aniline in water}}{C \text{ aniline in kresol}} + \text{const.}$$

would be therefore

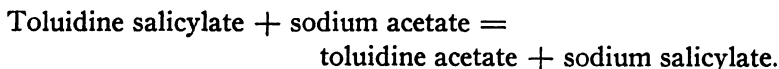
$$e = \frac{RT}{F} \log C \text{ aniline in water} + \text{const.}$$

If the aqueous concentration is changed 1 to 5, the change of the potential difference, e , would be 40. This condition is not quantitatively fulfilled, but a smaller change of the potential difference in the direction predicted by the theory is observed.

In an analogous way the change of the potential difference:



may be explained; the reaction which takes place in this case is:

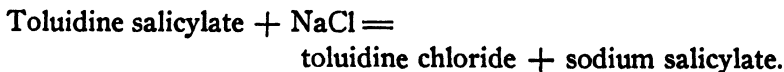


The "merely organic" salt, toluidine acetate, is more soluble in kresol, therefore the acetate anions aggregate in the kresol. The potential difference is determined in this case by the formula:

$$e = \frac{RT}{F} \log \frac{C \text{ acetate in water}}{C \text{ acetate in kresol}} + \text{const.}$$

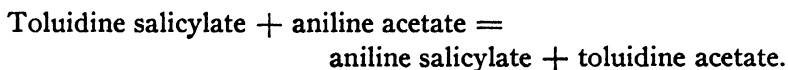
As " C acetate in kresol" is constant, or at least does not change proportionally to " C acetate in water," the potential difference e must vary in a reversible way with respect to anions, that is, in a direction opposite to that with aniline chloride as aqueous electrolyte.

We can also explain the action of a neutral salt like NaCl in this way; the reaction would be in this case:



Both products of the reaction are "half-organic" salts, and neither Na⁺ or Cl⁻ can aggregate therefore in the kresol: the potential difference must be independent of the concentration, as was really observed.

Finally, we will discuss the action of such a salt as aniline acetate. In this case our theory would seem to predict the following. The reaction would be:



In this case also both salts are distributed between kresol and water at equal rates, as they are both soluble in kresol according to our previous assumption. The potential difference, therefore, should be independent of the concentration. This was confirmed by experiment, as the following figures show:

TABLE VI.
Aniline Acetate Solution.
(Same system as Table V.)

Concentration	E M F. Millivolts
m/2	— 9
m/10	— 4
m/50	— 2

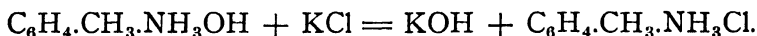
These theoretical explanations are incomplete owing to their qualitative character, since the distribution of the various salts is not known numerically; the writer has not yet been able to make such quantitative measurements. I wish to mention one qualitative experiment only, which shows the difference in distribution of aniline chloride and aniline acetate between water and kresol. If we shake an aqueous solution of aniline acetate with kresol, no aniline can be precipitated by KOH, which shows that the aniline has been taken up by the kresol; with an aqueous solution of aniline chloride, however, the aniline stays in the water after shaking with kresol under the same conditions, and can be identified by precipitation with KOH.

This theory may be applied to the electromotive forces generated by salicylic aldehyde or toluidine as follows: We take as an example the potential difference between salicylic aldehyde (saturated with salicylic acid) and KCl in aqueous solution.

The following reaction would take place in this case :



This reaction is very incomplete, *i. e.*, only very small amounts of $\text{C}_6\text{H}_4.\text{OH}.\text{COOK}$ and HCl are formed, but the $\text{C}_6\text{H}_4.\text{OH}.\text{COOK}$ is more soluble in the salicylic aldehyde, and, as KCl is present in the salicylic aldehyde in minimal traces only, the concentration of K ions in the aldehyde is chiefly due to $\text{C}_6\text{H}_4.\text{OH}.\text{COOK}$. This means that the concentrations of K ions in water and in the aldehyde are not proportional. Thus it is explained why the potential difference is reversible for cations. With a basic substance such as toluidine the opposite effect can be explained in the same way; the reaction may be in this case :⁹



$\text{C}_6\text{H}_4.\text{CH}_3.\text{NH}_3\text{Cl}$ formed in traces only aggregates in the toluidine, thus making the concentration of Cl ions in toluidine independent of the concentration of Cl ions in the aqueous solution. Evidently in this way the fact is explained that any cation affects the potential difference with salicylic aldehyde and any anion with toluidine. It is just this observation which appears strange at first and hard to explain from a theoretical standpoint.

As the relation between concentration and potential difference can be examined by experiment with a sufficient accuracy over a considerable range, I have also tried a quantitative application of my theory. A mathematical development appeared possible by means of the laws of distribution and of mass-action and the assumption that the concentration of H ions and the total concentration of salicylic acid in the salicylic aldehyde remained constant. A full account of this theory will be published later;¹⁰ it is remarkable that the larger variation of the potential difference at low concentration can be fully accounted for by this theory; as an example I wish to give here the values calculated for the change of the potential dif-

⁹ The formation of the compound $\text{C}_7\text{H}_8.\text{NH}_3.\text{OH}$ from $\text{C}_7\text{H}_8.\text{NH}_2$ and H_2O is analogous to the formation of NH_4OH .

¹⁰ Zeitschr. f. Electrochemie., 19, 1913.

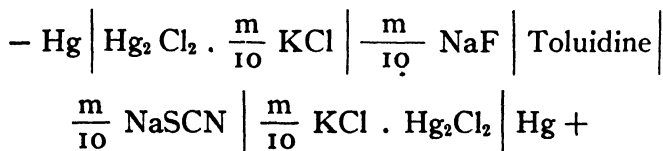
ference between toluidine and KSCN solution and compare them with the observed values:

TABLE VII.

Change of Concentration		Calculated	Change of E.M.F.	Observed
From 5/2	to 1/2m	19		26 (?)
" 1/2	" 1/10m	21		27
" 1/10	" 1/50m	27		30
" 1/50	" 1/250m	34		35
" 1/250	" 1/1250m	38		37
" 1/1250	" 1/6250m	40		42
" 1/6250	" 1/31250m	40		42

IV. ELECTROMOTIVE FORCES DUE TO CHEMICAL REACTION.

The E. M. F. of systems which are mere concentration cells, as those described above, is limited, since the range over which the concentration can be varied is limited. With toluidine as an electrode and KSCN as an aqueous electrolyte, for instance, the concentration could not be increased beyond $m/2$, because irreversible changes of the E. M. F. (due apparently to more complicated chemical changes) interfered in more concentrated solutions; neither could the concentration be decreased beyond $m/31,250$, because very irregular and rapidly varying changes of the E. M. F. were observed. With such low concentrations traces of other ions apparently interfere. Further, the total range over which the E. M. F. can be varied reversibly does not exceed 0.18 volt. With other systems the range was, as a rule, considerably smaller. As was said above, larger E. M. F.s can be produced easily by systems which contain solutions of different salts, for instance by the following system:



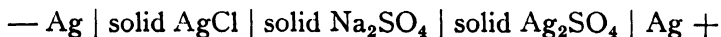
The E. M. F. is 0.23 volt.

While the E. M. F. of a concentration cell is due to merely physical equilibrium of two different concentrations, according to the well-known thermodynamic principles, the E. M. F. of a

system like that just mentioned is apparently caused by a chemical reaction.

The question presents itself how this reaction can be determined for a definite cell system. A solution is found if we make use of the principles which we have explained in the first part of my previous paper¹¹ concerning the calculation of the E. M. F.s of cells formed of solid salts.

As an example, I wish to discuss the last cell system. According to the previous explanations the toluidine contains toluidine fluoride in the region where it is in contact with the NaF solution, and it contains toluidine sulfocyanate in the region where it is in contact with NaSCN solution. A system of this kind is, therefore, analogous to the cell



discussed in the previous paper,¹¹ in the following respects:

1. Both systems are built up from four electrolytes containing two cations and two anions:

(1) AgCl, NaCl, Na₂SO₄, Ag₂SO₄.

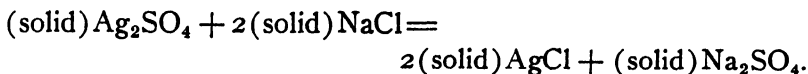
(2) NaF, Toluidine HF, Toluidine HSCN, KSCN.

2. The metallic electrodes are in both cases without influence upon the E. M. F.

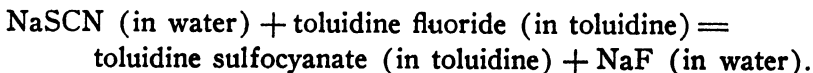
3. Both systems have electromotive forces at the junction of electrolytic phases.

The "toluidine" cell contains one phase junction less than the cell with the Ag salts; it can be proved, however, that this is without influence.

It was proved (*l. c.*) that the reaction is for the Ag cell:



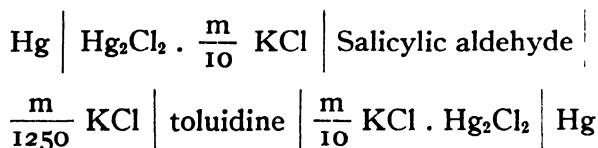
By analogy we find for the toluidine cell:



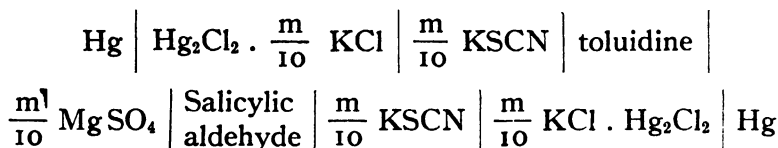
¹¹ Trans. Amer. Electrochem. Soc., **21**, 214 (1912).

It must be said, however, that it is not possible to calculate the magnitude or even the direction of the E. M. F. of the toluidine cell from these considerations since the concentration of the toluidine salts in the toluidine is entirely unknown. Besides, the thermal data have not been determined.

Considerable E. M. F. can also be produced by a system combining toluidine and salicylic aldehyde; for instance, a double concentration cell:



the E. M. F. of which is 0.19 volt, or:

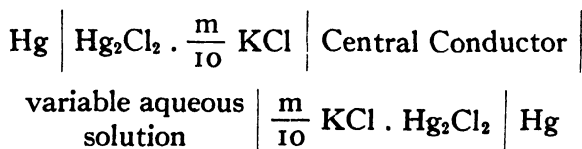


the E. M. F. of which is 0.34 volt.

V. SOME MEASUREMENTS OF THE E. M. F. OF SYSTEMS CONTAINING VARIOUS IMMISCIBLE ORGANIC LIQUIDS AS CENTRAL CONDUCTORS.

As was said above, the potential differences at "toluidine electrodes" can be imitated with various other water-immiscible organic bases. I wish to give here some measurements of this kind.

TABLE VIII.



(1) *Central Conductor: Aniline.*

Variable solution NaCl	E. M. F. Millivolts	Variable solution KCl	E. M. F. Millivolts
m/10	0	m/10	0
m/100	-21	m/100	-18
m/1000	-40	m/1000	-44

(2) Central Conductor: Mixture of 5 Vol. α -Naphthylamine and 2 Vol. Aniline.

Variable solution	E.M.F. Millivolts	Variable solution	E.M.F. Millivolts
m/10 NaCl	0	m/10 KCl	0
m/100 NaCl	-23	m/100 KCl	-39
m/1000 NaCl	-71	m/1000 KCl	-79

(3) Central Conductor: Monomethylaniline.

Variable solution	E.M.F. Millivolts	Variable solution	E.M.F. Millivolts
KCl		KSCN	
m/10	0	m/10	+151
m/50	-22	m/50	+118
m/250	-46	m/250	+85

(4) Central Conductor: Xylidine.

Variable solution	E.M.F. Millivolts
KCl	
m/10	0
m/250	-31
m/1250	-56

The measurements given in (3) show that the peculiar effect of SCN ions which was observed with toluidine is also produced with methylaniline. It seemed worth while to examine the effect of a sulfocyanate (causing a high potential) and of sulphates (causing a negative potential) with various bases. It may be remembered, again, that m/10 KCl is the arbitrary zero with all measurements.

TABLE IX.

(System same as Table VIII.)

(1) Methylaniline.

Concentration	E.M.F. Millivolts
m/1 MgSO_4	-40
m/10 KSCN	+145

(3) Aniline.

Concentration	E.M.F. Millivolts
m/10 KSCN	+84
m/10 Na_2SO_4	-141
m/10 MgSO_4	-137

(2) Xylidine.

m/10 KSCN	+124
m/10 Na_2SO_4	-69
m/10 MgSO_4	-92

(4) Naphthylamine and 1/10 Vol. Toluidine.

m/10 KSCN	+123
m/10 Na_2SO_4	-78

These data show that the sulfocyanates and sulphates act in the same way with all bases; the potential differences are, however, somewhat different in the different cases.

The question presented itself whether effects similar to those obtained with aromatic bases and aldehydes could also be produced with fatty compounds.

The alkyl substituted N-compounds are mostly miscible with water, and so are the lower fatty aldehydes.

As a water-immiscible fatty substance, the compound $\text{CH}_3\text{-(CH}_2)_5\text{-C-OH}$, the so-called oenanthic aldehyde, which is easily obtainable, was used. The aldehyde apparently contains a higher fatty acid due to oxidation, but no concentration effect similar to that of salicylic aldehyde could be produced, as the following figures show :

TABLE X.
Oenanthic Aldehyde.

Variable solution	E.M.F. (millivolts)
m/10 KCl	0
m/50 KCl	— 17
m/250 KCl	— 31
m/1250 KCl	— 17

As is seen, the potential difference varies in opposite direction to that with salicylic aldehyde from m/10 to m/250, but when the concentration is decreased still further the potential difference becomes more positive. Such a peculiar change of the potential difference in two directions was observed with a number of other substances, *e. g.*, phenol or benzaldehyde, if the aqueous electrolyte used was HCl. The explanation of this phenomenon seems to be rather difficult.

I wish to give here also my measurements with esters of lower fatty acids and alcohols.

TABLE XI.

Concentration KCl	Methyl Acetate E.M.F.(m.v.)	Ethyl Acetate E.M.F.(m.v.)	Butyl Acetate E.M.F.(m.v.)
m/2	..	..	— 24
m/10	0	0	0
m/50	..	+ 10	+ 29
m/250	..	+ 31	+ 60
m/1250	0	+ 50	+ 79

In the two last series of measurements the reversibility was unsatisfactory; the potential difference underwent irregular changes

when the concentration of KCl was decreased, starting from $m/2$ concentration.

It is remarkable that the change of potential difference is larger with the higher esters, probably owing to the increasing immiscibility with water.

The following measurements with ethyl and amyl ester showed a fair reversibility:

TABLE XII.

Concentration of KCl	Ethyl Butyrate E.M.F.(m.v.)	Ethyl Valerate E.M.F.(m.v.)	Amyl Acetate E.M.F.(m.v.)	Aceto-acetic Ester E.M.F.(m.v.)
5 $m/2$	..	..	..	— 12
$m/2$	..	..	..	— 6
$m/10$	0	..	+ 1	0
$m/50$	+ 18	+ 4	— 1	+ 11
$m/250$	+ 38	+ 42	+ 36	+ 28
$m/1250$	+ 66	+ 88	+ 52	+ 52
$m/6250$	..	..	..	+ 76

It is seen from these measurements that the potential difference at the junction of esters and KCl solutions becomes more positive with decreasing concentration; the change is greater and more perfectly reversible with the esters which contain a great number of C-atoms.

As proved above, the acid contained in the second phase is mainly responsible for the change of the potential difference in this direction. One can conclude that the acid which is always contained in the esters (being formed through saponification) causes the effect in the last cases. This is also seen from the fact that benzonitrile, $C_6H_5.CN$, a substance of very different constitution, acts also as an electrode for various cations, the change of the potential difference being in the same direction. Apparently the benzoic acid formed by saponification is the cause. The following measurements with benzonitrile prove this:

TABLE XIII.

Benzonitrile.

Concentration	E.M.F.(m.v.)
$m/10$ KCl	0
$m/50$ KCl	+ 34
$m/250$ KCl	+ 65
$m/1250$ KCl	+ 84

It was also found that the changes of potential were greater when the benzonitrile had been allowed to be in contact with water for some hours, thus producing a larger amount of benzoic acid.

The theoretical explanation would seem to be the same as with salicylic aldehyde in all cases. However, the reversibility is not always satisfactory, as was mentioned. We can only guess which circumstances cause the irreversibility (*e. g.*, with methyl acetate); there may be a formation of unstable compounds in the boundary regions which interferes.

DIE URSACHE DER SPONTANEN FURCHUNG BEIM UNBEFRUCHTETEN SEEIGELEI.*

VON JACQUES LOEB.

(Aus den Laboratorien des Rockefeller Institute for Medical Research,
New York.)

MIT 13 FIGUREN IM TEXT.

1) Wir haben bisher keine Antwort auf die Frage, wie es kommt, dass die Eier gewisser Arten imstande sind, sich ohne Samen zu entwickeln, während bei den Eiern der meisten Formen das Eindringen eines Spermatozoons (oder der Ersatz dieses Vorganges durch die Methoden der künstlichen Parthenogenese) die unerlässliche Voraussetzung der Entwicklung ist. Allein auch bei diesen Eiern ist gelegentlich eine Tendenz vorhanden, wenigstens die Anfangsstadien der Entwicklung zu durchlaufen. Eine solche Tendenz ist bei den Eiern des Seesterns häufiger und ausgesprochener als bei denen des Seeigels. Bei den Eiern von *Asterias Forbesii* in Woods Hole kann es zu weitgehenden spontanen Furchungen und sogar Larvenbildungen kommen, namentlich wenn man die Eier leicht schüttelt. Bei den Eiern des Seeigels kommt es nicht zu spontanen Larvenbildungen, auch nicht, wenn man die Eier schüttelt. Wenn aber die Eier gewisser Seeigel längere Zeit in Seewasser liegen, so bemerkt man gelegentlich eine Tendenz dieser Eier sich zu furchen. Das ist namentlich beim Ei von *Arbacia* der Fall. Die Furchung ist meist nicht regelmässig, und geht selten über das 2- oder 4-Zellstadium hinaus, worauf die Eier zugrunde gehen. Diese spontane Furchung ist nicht bei den Eiern aller Weibchen zu beobachten. Bei den Eiern von *Strongylocentrotus purpuratus* ist die spontane Furchung unbefruchteter Eier ungemein selten. Was bedingt nun diese spontane Furchung unbefruchteter Eier?

* Eingegangen am 29. Januar 1913.

Meine Versuche über künstliche Parthenogenese haben gezeigt, dass der Anstoss zur Entwicklung in einer Modifikation der Oberfläche des Eies besteht, die oft, aber nicht immer, zur Membranbildung führt. Ich will im folgenden einige Beobachtungen mitteilen, welche zeigen, dass die spontane Furchung bei solchen Eiern vorkommt, bei denen eine spontane Tendenz zur Membranbildung vorhanden ist. Diese Tendenz ist offenbar nur bei den Eiern gewisser Weibchen vorhanden. Die Versuche wurden an den Eiern von *Strongylocentrotus purpuratus* in Pacific Grove angestellt. Der Beschreibung der Versuche möchte ich einige Bemerkungen über den Vorgang der Membranbildung vorausschicken.

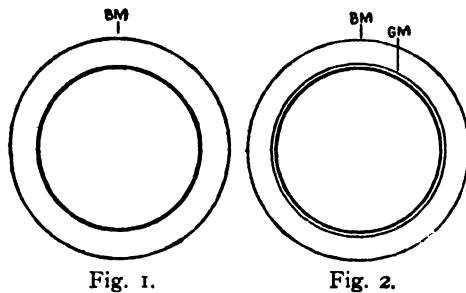


Fig. 1.

Fig. 2.

Fig. 1. Befruchtungsmembran *BM* unmittelbar nach dem Eindringen eines Spermatozoons oder der künstlichen Membranbildung durch Buttersäure.

Fig. 2. Dasselbe Ei etwa eine halbe Stunde später. Inzwischen hat sich auf dem Cytoplasma die gelatinöse Auflagerung oder Membran *GM* gebildet. Durch Zusatz von etwas KCN zum Seewasser wird die Bildung dieser Membran verzögert oder unterdrückt.

Wenn ein Spermatozoon in das Ei von *Strongylocentrotus purpuratus* eindringt, oder wenn wir die Membranbildung bei diesem Ei mit Buttersäure hervorrufen, so liegt bei Eiern, die eben aus den Ovarien entnommen sind, die Membran in weitem Abstand vom Ei (Fig. 1). Die Membran ist für Wasser, Zucker und Salze durchgängig, für Kolloide (z. B. Eiweiss) undurchgängig. Der Raum zwischen Membran und Ei ist mit Flüssigkeit, und zwar wesentlich Seewasser, gefüllt, was, wie ich schon früher zeigte¹⁾, dadurch beweisbar ist, dass Zusatz irgendeiner Kolloidsubstanz zum See-

¹⁾ J. LOEB, Über die osmotischen Eigenschaften und die Entstehung der Befruchtungsmembran beim Seeigelei. Arch. f. Entw.-Mech. Bd. 26. 1908. S. 82.

wasser die Membran zum Kollabieren bringt (Fig. 3—6). Bringt man das Ei in reines Seewasser zurück, so stellt sich sofort die Membran wieder her, wie in Fig. 1. Ich habe mich überzeugt, dass dieser Versuch schon unmittelbar nach der Entstehung der Membran so verläuft, was beweist, dass bei diesem Ei der Raum zwischen Membran und Ei von vornherein nur aus Flüssigkeit besteht.

Bald nach Bildung der Befruchtungsmembran *BM* bildet sich eine helle, gelatinartige Auflagerung auf dem Eiprotoplasma (*GM* Fig. 2). Die Bildung dieser Auflagerung erfolgt langsam und scheint von den im Ei stattfindenden Oxydationen abzuhängen.

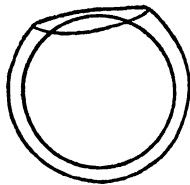


Fig. 3.

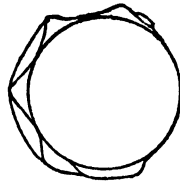


Fig. 4.

Fig. 3 und 4. Partieller Collaps der Befruchtungsmembran auf Zusatz einer kleinen Menge von Eiweiss zum Seewasser. Es bilden sich Dellen und die Membran legt sich stellenweise dem Cytoplasma dicht an, so dass man glauben könnte, dieselbe sei aufgelöst.

Fügt man zu 50 ccm Seewasser nämlich 4 bis 6 Tropfen $\frac{1}{10}$ Prozent KCN zu und bringt man die Eier unmittelbar nach dem Eindringen des Spermatozoons oder nach der Behandlung mit Butter-säure in dieses cyankaliumhaltige Seewasser, so bildet sich die Befruchtungsmembran, aber die Bildung der inneren, dem Protoplasma aufliegenden hellen Schicht wird verzögert, oder gelegentlich lange Zeit ganz unterdrückt.

Wenn wir Eier befruchten, welche bei Zimmertemperatur 24 Stunden oder länger in Seewasser gelegen haben, so wird anscheinend keine Befruchtungsmembran gebildet. In Wirklichkeit wird wohl eine Membran gebildet, die aber dem Cytoplasma dicht anliegt. Ferner wird möglicherweise die gelatinöse Schicht gebildet, welche nachträglich (durch Oxydation im Protoplasma?) an der Oberfläche des letzteren entsteht. Es fehlt also dem Ei in diesem Falle die Schicht von Seewasser zwischen der Befruchtungsmembran *BM* und der gelatinösen Schicht *GM* (Fig. 2), welche dem Protoplasma

auffliegt. Möglicherweise ist auch die Befruchtungsmembran viel dünner und feiner als in einem Ei, das gleich nach dem Herauskommen aus dem Ovarium befruchtet wird.

2) Wenn man unbefruchtete Eier von *Strongylocentrotus* in Seewasser bei einer relativ niederen Temperatur aufbewahrt, so beobachtet man, dass nach etwa 24 bis 48 Stunden bei einem Teil der Eier gewisser Weibchen eine Membranbildung auftritt, wie sie am Ende des vorigen Abschnitts beschrieben wurde; d. h. es bildet sich ein heller, gelatinartiger Belag um die Eier, aber ohne dass die Befruchtungsmembran durch eine dicke Flüssigkeitsschicht vom Protoplasma getrennt ist¹⁾. Bringt man solche Eier in ein Gefäss, in

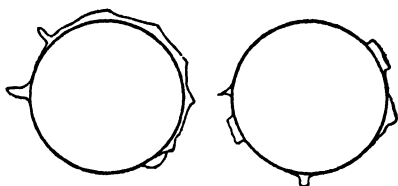


Fig. 5.

Fig. 6.

Fig. 5 und 6. Collaps der Membran beim Zusatz von mehr Eiweiss zum Seewasser. Fast alle Flüssigkeit diffundiert infolge des osmotischen Überdruckes im umgebenden Seewasser aus dem Membranraum und die Membran legt sich dem Cytoplasma dicht an. Bei Übertragung der Eier in eiweissfreies Seewasser stellt sich die normale Befruchtungsmembran sofort wieder her.

dem sie nicht in dichten Haufen liegen, und hält man dieselben bei niedriger Temperatur, so fangen dieselben an, sich zu furchen, und diese Furchung kann gelegentlich regelmässig erfolgen und — bei niedrigerer Temperatur — bis zum 8- oder 16-Zellstadium fortschreiten (Fig. 7 bis 13). Ich glaubte anfangs, dass zufällig Samen in diese Kulturen geraten sei, überzeugte mich aber, durch Wiederholung der Versuche, dass das nicht der Fall war. Denn diese Eier entwickeln sich nie weiter als bis etwa zum 16-Zellstadium, während die mit Samen befruchteten Eier sich unter denselben Umständen bis zu Pluteen entwickeln. Nur die Eier, welche spontan eine Mem-

¹⁾ Das dürfte wohl daher rühren, dass der Membranbildungsprozess erst dann eintritt, wenn die Eier schon lange im Seewasser gelegen haben. Unter diesen Umständen liegt auch die Befruchtungsmembran, welche durch ein Spermatozoon gebildet wird, dem Ei meist dicht an.

bran bilden, furchen sich. In der Membranbildung selbst zeigen sich Unterschiede. Oft bleibt die Membran während der Furchung erhalten, manchmal aber ist dieselbe sehr zart und zerreisst während der Furchung, oder fällt ab bei der Übertragung der Eier aus einem Gefäss in ein anderes. Die Zahl der Weibchen, deren Eier spontan eine Membran bilden, ist nicht sehr gross, und sie beträgt vielleicht nicht mehr als etwa 5 Prozent. Nicht alle Eier solcher Weibchen bilden Membranen, sondern nur ein variierender Prozentsatz, der auch meist klein ist.

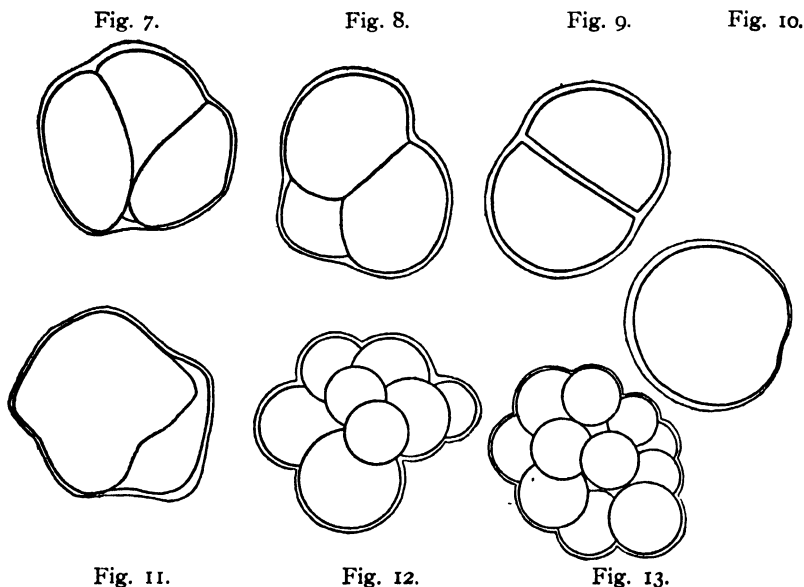


Fig. 7 bis 13. Spontane Membranbildung und Furchung bei unbefruchteten Eiern, welche über 48 Stunden bei einer Temperatur von 4° bis 8° C. gehalten waren. Als die Eier der Zimmertemperatur ausgesetzt wurden, gingen sie sofort zugrunde. Auch bei niedriger Temperatur entwickelten sie sich nicht weiter.

Wäre es möglich, die unbefruchteten Eier von Seeigeln länger am Leben zu erhalten, so würden auf die Dauer möglicherweise die Eier vieler, vielleicht aller, Weibchen eine spontane Membranbildung mit darauf folgender Furchung zeigen. Bei der kurzen Lebensdauer der unbefruchteten Eier in Seewasser beobachten wir diese spontane Membranbildung nur bei den Eiern weniger Weibchen. Die Eier, welche keine Membran bilden, furchen sich auch nicht.

3) Wir wollen nun zeigen, dass die Eier, welche spontan eine Membran bilden, sich ebenso verhalten wie die Eier, bei denen die Membranbildung künstlich durch Buttersäurebehandlung hervorgerufen wird. Dieser Nachweis kann durch zwei Tatsachen geführt werden.

Wenn man bei den unbefruchteten Eiern von *Strongylocentrotus purpuratus* die künstliche Membranbildung durch Buttersäure hervorruft und die Eier bei Zimmertemperatur hält, so fangen sie bekanntlich an, sich zu furchen, gehen aber meist schon bei der ersten oder zweiten Teilung zugrunde. Hält man sie bei niedriger Temperatur, so können sie eine oder eine Reihe von Teilungen durchmachen, ehe sie zerfallen. Genau so verhalten sich die Eier, welche spontan eine Membran bilden und sich spontan furchen. Bei niedriger Temperatur furchen sie sich bis zum 16-Zellstadium, bei Zimmertemperatur zerfallen sie nach der ersten Furchung. Bei den Eiern von *Arbacia*, die sich im Hochsommer in Woods Hole entwickeln, habe ich nie eine spontane Furchung über 2 oder 3 Zellen hinaus beobachtet, vermutlich wegen der hohen Temperatur.

Zweitens können die Eier nach der künstlichen Membranbildung durch Buttersäure auch bei Zimmertemperatur veranlasst werden, sich zu normalen Larven zu entwickeln, wenn man dieselben nach der Membranbildung 30 bis 60 Minuten in hypertonisches Seewasser bringt (50 ccm Seewasser + 8 ccm $2\frac{1}{2}$ m NaCl). Dasselbe gilt für die Eier, welche spontan eine Membran bilden und sich spontan furchen. Eier eines Weibchens, welche spontan Membranen bildeten, wurden in hypertonisches Seewasser gebracht, und nach 35, 40, 50 und 60 Minuten in Seewasser übertragen. Die Temperatur betrug etwa 15°. Unter den nach 60 Minuten übertragenen Eiern bildete sich eine kleine Zahl Larven, aber nur solche Eier entwickelten sich zu Larven, welche eine Membran gebildet hatten. Zur Kontrolle wurden die Eier eines Weibchens, die keine Tendenz zur Membranbildung zeigten, ebenfalls 30 bis 60 Minuten in das hypertonische Seewasser gebracht. Kein Ei entwickelte sich. Das entspricht allen früheren Versuchen in dieser Richtung.

Die spontane Membranbildung hat also dieselbe entwicklungs-erregende Bedeutung für das Ei von *Strongylocentrotus*, wie die künstliche Anregung der Membranbildung durch Buttersäure.

4) Wir müssen die Frage aufwerfen, was die anscheinend spontane Membranbildung der Eier gewisser Weibchen nach längerem Liegen im Seewasser bedingt. Darauf können wir einstweilen keine Antwort geben, sondern nur die Vermutung ausdrücken, dass es wohl durch eine in der Lösung vorhandene Substanz, etwa NaOH geschieht.

Für diese Vermutung ergibt sich eine Stütze in der Tatsache, dass die Entwicklungserregung durch Basen, selbst durch NH_4OH^1), nicht bei den Eiern aller Weibchen von *purpuratus* gelingt. Da die Voraussetzung der künstlichen Parthenogenese die Modifikation der Oberfläche des Eies, d. h. eine Membranbildung ist, so beweist das, dass Basen nicht bei allen Weibchen von *Strongylocentrotus purpuratus* die Membranbildung hervorzurufen imstande sind.

Was die parthenogenetische Furchung des Seesterneies betrifft, so ist dieses Ei dadurch ausgezeichnet, dass es bei ihm spontan leicht zur Membranbildung kommt, oder dass leichtes Schütteln diese Membranbildung hervorruft. Daher ist auch hier die spontane Furchung häufiger als beim Ei des Seeigels.

ZUSAMMENFASSUNG DER RESULTATE.

Es wird gezeigt, dass die spontane Furchung, welche gelegentlich bei Seeigeleiern nach längerem Liegen beobachtet wird, durch eine spontane Membranbildung dieser Eier bedingt ist. Es scheint, dass die Eier gewisser Weibchen nach längerem Liegen in Seewasser veranlasst werden, eine Membran zu bilden. Solche Eier verhalten sich ähnlich wie Eier, bei denen die künstliche Membranbildung durch Buttersäure hervorgerufen ist. Durch eine kurze Behandlung mit hypertonischem Seewasser werden sie veranlasst, sich zu

¹⁾ Ich habe neuerdings gefunden, dass die schwachen Basen wie NH_3 und gewisse Amine viel wirksamer für die künstliche Parthenogenese sind, als die starken Basen wie NaOH oder Tetraäthylammoniumhydroxid. Einige dieser Versuche sind schon veröffentlicht (Journal of Experimental Zoology. Vol. 13. 1912. p. 577). Diese Tatsache ist das Analogon meiner im Jahre 1905 mitgeteilten Beobachtung, dass die schwachen Säuren, wie Fettsäuren, CO_2 , viel geeigneter für die künstliche Parthenogenese sind als die starken Säuren, wie HCl oder Oxalsäure. Die für die Säure gegebene Erklärung, nämlich dass die schwachen Säuren leichter in das Ei eindringen und deshalb wirksamer sind, gilt wohl auch für das entsprechende Verhalten der Basen.

Larven zu entwickeln; ohne eine solche Behandlung gehen sie bei Zimmertemperatur während oder nach der ersten Furchung zugrunde, während sie bei niederer Temperatur sich etwas weiter furchen können.

Die Beobachtungen liefern so eine weitere Stütze für die Annahme, dass die Veränderungen der Oberfläche des Eies, welche der Membranbildung zugrunde liegen, die Voraussetzung für die Entwicklung des Eies bilden.

Die Versuche wurden in meinem alten Laboratorium in Pacific Grove angestellt und ich möchte meinen früheren Kollegen, Prof. MAXWELL, MOORE und ROBERTSON, für ihre Freundlichkeit meinen verbindlichsten Dank aussprechen.

DIE BEDEUTUNG DER LIPOIDE FÜR DIE ENT- STEHUNG DER BIOELEKTRISCHEN POTEN- TIALDIFFERENZEN BEI GEWISSEN PFLANZLICHEN ORGANEN.*

VON JACQUES LOEB UND REINHARD BEUTNER.

*(Aus den Laboratorien des Rockefeller Institute for Medical Research,
New York.)*

1. Es schien von Interesse, die Stoffe kennen zu lernen, die die bioelektrischen Erscheinungen bestimmen. Diese Aufgabe konnte ausgeführt werden, da wir in den vorausgehenden Arbeiten die sehr charakteristischen Eigentümlichkeiten der Potentialdifferenzen an der Grenze pflanzlicher und tierischer Organe und den Zuleitungsflüssigkeiten kennen gelernt haben¹⁾).

Wir gehen von folgender Kette aus:

Hg | HgCl m-KCl | lebendes Organ | KCl (variable Konzen-
tration) | m-KCl HgCl | Hg.

Eine zunehmende Verdünnung der variablen KCl-Lösung macht die rechte Seite positiver. Das gilt nicht nur für KCl, sondern für jede wässrige Lösung eines Alkali- und Erdalkalisalzes. Die bioelektrischen Potentialdifferenzen sind also reversibel für Kationen jeder Art. Wasserlösliche Säuren sind negativ im Vergleich mit wasserlöslichen Salzen der gleichen Konzentration, und zwar ist die Potentialdifferenz von der Grössenordnung der EMK des Verletzungsstromes.

Die Grösse der Änderung der Potentialdifferenz mit der Änderung der Konzentration der Zuleitungsflüssigkeit ist in einem grossen Bereich der Konzentration beinahe so gross, wie man nach der Nernstschen Formel für Potentialdifferenzen erwarten sollte.

* Eingegangen am 23. April 1913.

¹⁾ Loeb und Beutner, diese Zeitschr. 41, 1; 44, 303, 1912.

Ferner beobachteten wir, dass die bioelektrische Potentialdifferenz mit steigender Konzentration einem Grenzwert zustrebt. Beutner hat für diese Vorgänge eine Theorie entwickelt, wonach dieselben dadurch zustande kommen, dass Spuren der Elektrolyte der Lösung mit einem Elektrolyten der wasserunlöslichen Phase chemische Reaktionen eingehen. Er hat nachweisen können, dass an der Grenze von wasserunlöslichen Säuren (z. B. Salicylsäure in Salicylaldehyd gelöst) und wässerigen Lösungen Potentialdifferenzen auftreten, die eine ähnliche Abhängigkeit von dem Konzentrations-einfluss zeigen wie pflanzliche und tierische Organe¹).

Es fragte sich nun, welche Stoffe in den lebenden Organen die bioelektrischen Potentialdifferenzen und die vorhin geschilderten Eigentümlichkeiten derselben unter verschiedenen Bedingungen bestimmen.

2. Versuche mit Eiweisskörpern. Die erste Möglichkeit, die sich bot, war die, dass feste Eiweisskörper die wasserunlösliche Phase bilden. Diese Möglichkeit wurde an fester Gelatine und an geronnenem Hühnereiweiss geprüft. Das Resultat war völlig negativ. Die Versuchsanordnung war dieselbe wie bei unseren früheren Versuchen, nur dass die festen Eiweisskörper die Stelle der lebenden Organe in der Kette einnahmen.

Kette:

Hg | HgCl^m/₁₀-NaCl | festes Eiweiss | NaCl (variable Konzentration) | ^m/₁₀-NaCl HgCl | Hg.

TABELLE I.

Potentialdifferenz an der Grenze von geronnenem Eiweiss und NaCl verschiedener Konzentration.

Konzentration der NaCl-Lösung	EMK Volt
^m / ₁₀	-0,002
^m / ₁₀₀₀	-0,005
^m / ₁₀₀	-0,008
^m / ₁₀	-0,005

Wie man sieht, fehlt jeder Konzentrationseinfluss, der für lebende Organismen charakteristisch ist, nicht einmal das Positivwerden mit abnehmender Konzentration tritt in die Erscheinung.

¹) Beutner, diese Zeitschr. 47, 73, 1912; Zeitschr. f. Elektrochem. 19, 1913.

Ebenso resultatlos verliefen die Versuche mit Gelatine. Wir dürfen also sagen, dass die festen Eiweisskörper nicht die wasserunlösliche Phase bei der Entstehung der bioelektrischen Potentialdifferenzen bilden.

3. Versuche mit Lecithin. Die Versuche mit Lecithin ergaben eine überraschende Übereinstimmung mit denen am Apfel oder dem Blatt der Gummipflanze.

Es war nötig, das Lecithin (wie auch die anderen weiterhin zu besprechenden Lipide) in ein Lösungsmittel zu bringen, um die Phasengrenzkräfte zu beobachten; da ja das Lecithin eine schmierige Masse ist, mit der sich nicht gut eine kontinuierliche trennende Oberfläche herstellen lässt. Als Lösungsmittel diente meist Guajacol. Die Methode war die gleiche wie die in Beutners früheren Versuchen beschriebene. Dieselbe bestand darin, dass der kleinere weite Schenkel eines U-Rohres mit Guajacol gefüllt wurde, während der längere Schenkel mit m-KCl gefüllt war und mit der Elektrode (bestehend aus Hg HgCl $m/1$ -KCl) verbunden war. Der kleine Schenkel mit Guajacol wurde nacheinander in KCl-Lösungen verschiedener Konzentration gebracht und die EMK festgestellt.

Kette:

Hg | HgCl $m/1$ -KCl | Guajacol | KCl (variable Konzentration) | m-KCl HgCl | Hg.

TABELLE II.

Reines Guajacol.

Konzentration der variablen KCl-Lösung	EMK Volt
$m/1$	+0,002
$m/1000$	+0,044
$m/100$	+0,027
$m/10$	+0,006
m	+0,001

Das Guajacol zeigt also schon einen Konzentrationseinfluss in demselben Sinne wie die lebenden Organe. Dasselbe ist also auch für Kationen reversibel. Der Konzentrationseinfluss ist aber erheblich kleiner als der beim Apfel gefundene. Dagegen zeigt das reine Guajacol nicht den Säureeinfluss, der für den Apfel charakter-

istisch ist, nämlich, dass, wenn wir mit wässriger Säurelösung und Salzlösung ableiten, die Säure ein kleineres Potential ergibt.

Beispiel. Reines Guajacol.

$$\begin{array}{lcl} m/_{10}\text{-KCl} & . & . & . & . & -0,001 \text{ Volt} \\ m/_{10}\text{-HCl} & . & . & . & . & +0,012 \text{ "} \end{array}$$

Es besteht also hier ein kleiner Effekt im entgegengesetzten Sinne, wie beim Apfel oder dem Blatt der Gummipflanze.

Wir stellten nun eine 10⁰/₀ige Lösung des Merckschen käuflichen Lecithins in Guajacol her. Die Kette war dieselbe wie bei den Versuchen mit reinem Guajacol, nur dass 10⁰/₀ Lecithin im Gemisch gelöst war.

TABELLE III.

10⁰/₀ Lecithin, gelöst in Guajacol.

Konzentration der Lösung	EMK Volt	Differenz Volt
$m/_{1}\text{-KCl}$	+0,012	> 0,029
$m/_{10}\text{-KCl}$	0,041	> 0,050
$m/_{100}\text{-KCl}$	0,091	> 0,046
$m/_{1000}\text{-KCl}$	0,137	
$m/_{10}\text{-KCl}$	+0,042	> 0,042
$m/_{10}\text{-HCl}$	±0,000	
$m/_{1250}\text{-HCl}$	+0,091	> 0,039
$m/_{250}\text{-HCl}$	+0,052	> 0,037
$m/_{50}\text{-HCl}$	+0,015	> 0,021
$m/_{10}\text{-HCl}$	-0,006	

Diese Werte sind nun in fast allen Stücken identisch mit den Werten, die wir beim Apfel oder dem Blatt von *Ficus elasticus* unter den gleichen Bedingungen erhielten. Einer 10fachen Verdünnung von KCl entspricht eine Zunahme der EMK um ungefähr 0,050 Volt. Beim Apfel war sie in demselben Falle und in demselben Konzentrationsbereich 0,051 Volt. Zweitens finden wir auch hier, dass mit zunehmender Konzentration der Salzlösung die EMK einem maximalen Wert zustrebt. Endlich finden wir, dass auch hier bei Ableitung mit wässriger Säurelösung und Salzlösung gleicher Konzentration die Säure eine kleinere EMK gibt; und zwar ist der Unterschied ebenfalls von der Grössenordnung des Verletzungsstromes, wie beim Apfel: Wir können also die bioelek-

trischen Potentialdifferenzen beim Apfel mit einer Mischung von Lecithin in Guajacol qualitativ und quantitativ nachahmen.

Wir untersuchten nun das Verhalten einer 10⁰/₀igen Lecithinlösung in m-Kresol, um uns zu überzeugen, dass die gefundene Übereinstimmung nicht nur für ein einziges Lösungsmittel gilt. Gleichzeitig wollten wir uns noch überzeugen, ob diese Lösung in Bezug auf jedes Kation reversibel ist. Um zu zeigen, dass der gefundene Konzentrationseinfluss gleich demjenigen ist, den wir am lebenden Blatt der Gummipflanze gefunden haben, stellen wir die Resultate nebeneinander.

TABELLE IV.

Salzlösung	10% Lecithin in m-Kresol		Blatt der Gummipflanze	
	EMK Volt	Differenz Volt	EMK Volt	Differenz Volt
$\frac{m}{10}$ -KCl	+0,050			
$\frac{m}{250}$ -KCl	+0,150			
$\frac{m}{1250}$ -KCl	+0,141		0,099	
$\frac{m}{250}$ -KCl	+0,118	> 0,023	0,068	> 0,031
$\frac{m}{50}$ -KCl	+0,084	> 0,034	0,036	> 0,032
$\frac{m}{10}$ -KCl	+0,049	> 0,035	0,012	> 0,024
m-KCl	+0,018	> 0,031		
$\frac{m}{10}$ -KCl	+0,043			
$\frac{m}{10}$ -NaCl	+0,064			
$\frac{m}{1250}$ -NaCl	+0,150		0,141	
$\frac{m}{250}$ -NaCl	+0,128	> 0,022	0,103	> 0,038
$\frac{m}{50}$ -NaCl	+0,098	> 0,030	0,067	> 0,036
$\frac{m}{10}$ -NaCl	+0,061	> 0,037	0,043	> 0,024
$\frac{m}{2}$ -NaCl	+0,030	> 0,031		
$\frac{m}{10}$ -KCl	0,051			
$\frac{m}{10}$ -CaCl ₂	0,066			
$\frac{m}{1250}$ -CaCl ₂	0,126		0,116	
$\frac{m}{250}$ -CaCl ₂	0,101	> 0,025	0,085	> 0,031
$\frac{m}{50}$ -CaCl ₂	0,083	> 0,018	0,065	> 0,020
$\frac{m}{10}$ -CaCl ₂	0,065	> 0,018	0,053	> 0,012
$\frac{m}{2}$ -CaCl ₂	0,045	> 0,020		
$\frac{m}{10}$ -KCl	0,042			
$\frac{m}{2}$ -Na ₂ SO ₄	0,030			
$\frac{m}{250}$ -Na ₂ SO ₄	0,122			
$\frac{m}{50}$ -Na ₂ SO ₄	0,090	> 0,032		
$\frac{m}{10}$ -Na ₂ SO ₄	0,055	> 0,035		
$\frac{m}{2}$ -Na ₂ SO ₄	0,030	> 0,025		

Die Analogie im Verhalten des in m-Kresol gelösten Lecithins und des Blattes der Gummipflanze ist schlagend. Es zeigt sich

auch, dass das in Kresol gelöste Lecithin in Bezug auf jedes beliebige Kation reversibel ist. Hiervon muss die elektromotorische Wirkung des reinen m-Kresols abgezogen werden. Dieselbe ist aber noch geringer als die des reinen Guajacols, so dass die hier geschilderten Wirkungen fast ausschliesslich dem Lecithin zuzuschreiben sind.

4. Versuche mit Kephalin. Herr Dr. P. A. Levene war so freundlich, uns etwas chemisch reines Kephalin zu überlassen. Dasselbe ist relativ wenig löslich in Guajacol, und wir arbeiteten deshalb mit einer gesättigten Lösung in Guajacol.

TABELLE V.
Kephalin in Guajacol.

Salzlösung	EMK Volt	Differenz Volt
m-KCl	$\pm 0,000$	
$\frac{m}{10}$ -KCl	+0,038	
$\frac{m}{1250}$ -KCl	+0,141	
$\frac{m}{250}$ -KCl	+0,116	> 0,025
$\frac{m}{80}$ -KCl	+0,075	> 0,041
$\frac{m}{10}$ -KCl	+0,040	> 0,035
$\frac{m}{10}$ -NaCl	+0,066	
$\frac{m}{1250}$ -NaCl	+0,178	
$\frac{m}{250}$ -NaCl	+0,135	> 0,043
$\frac{m}{80}$ -NaCl	+0,096	> 0,039
$\frac{m}{10}$ -NaCl	+0,060	> 0,036
$\frac{m}{2}$ -NaCl	+0,027	> 0,033
$\frac{5m}{2}$ -NaCl	$\pm 0,000$	> 0,027
$\frac{m}{2}$ -NaCl	+0,026	
$\frac{m}{10}$ -NaCl	+0,065	

Das Kephalin weicht aber in zwei Punkten vom Lecithin ab. Erstens fehlt die negativierende Wirkung der Säuren, und zweitens ist die Calciumwirkung irreversibel.

$\frac{m}{10}$ -CaCl ₂	+0,073	
$\frac{m}{1250}$ -CaCl ₂	+0,129	
$\frac{m}{250}$ -CaCl ₂	+0,116	
$\frac{m}{80}$ -CaCl ₂	+0,074	
$\frac{m}{10}$ -CaCl ₂	+0,034	
$\frac{m}{10}$ -KCl	+0,027	
$\frac{m}{10}$ -HCl	+0,052	

Im übrigen aber verhält sich Kephalin wie Lecithin.

5. Da wasserunlösliche Säuren, wie Beutner¹⁾ gefunden hat, die Eigenschaft besitzen, für Kationen reversibel zu sein, so lag es

¹⁾ Beutner, Zeitschr. f. Elektrochemie 19, 1913.

nahe, die wasserunlöslichen Fettsäuren, wie Ölsäure, Stearinsäure, Palmitinsäure u. a., die in den Phosphatiden durch Zersetzung vorkommen, auf ihr elektromotorisches Verhalten zu untersuchen. Es wäre ja denkbar, dass das Verhalten des Lecithins durch seinen Gehalt an wasserunlöslicher Fettsäure bestimmt sei. Es stellte sich heraus, dass diese Säuren sich in der Tat wie Lecithin verhalten.

TABELLE VI.
10 Vol.-% Ölsäure in Guajacol.

Salzlösung	EMK Volt	Differenz Volt
$m/_{10}$ -KCl	+0,031	
$m/_{1250}$ -KCl	+0,124	> 0,030
$m/_{250}$ -KCl	+0,094	> 0,032
$m/_{50}$ -KCl	+0,062	> 0,028
$m/_{10}$ -KCl	+0,034	> 0,028
$m/_{5}$ -KCl	+0,006	> 0,006
$5m/_{2}$ -KCl	± 0,000	
$m/_{10}$ -KCl	+0,031	
$m/_{10}$ -CaCl ₂	+0,080	
$m/_{1250}$ -CaCl ₂	+0,136	> 0,026
$m/_{250}$ -CaCl ₂	+0,110	> 0,015
$m/_{50}$ -CaCl ₂	+0,095	> 0,016
$m/_{10}$ -CaCl ₂	+0,079	
$m/_{10}$ -KCl	+0,031	
$m/_{10}$ -HCl	+0,028	

Die Ölsäure verhält sich also wie das Lecithin und die lebenden Pflanzenorgane; eine Ausnahme bildet nur ihr Verhalten den Säuren gegenüber, wenigstens in gewissen Lösungsmitteln. Die bei Lecithin und den lebenden Organen beobachtete Erniedrigung des Potentials fehlt hier.

TABELLE VII.

Salzlösung	EMK Volt	Differenz Volt
Palmitinsäure in Guajacol.		
$1/_{1}$ -KCl	+0,022	
$1/_{1000}$ -KCl	+0,072	> 0,022
$1/_{100}$ -KCl	+0,050	> 0,023
$1/_{10}$ -KCl	+0,027	> 0,005
$1/_{1}$ -KCl	+0,022	

TABELLE VII (Fortsetzung).

Salzlösung	EMK Volt	Differenz Volt
Palmitinsäure in Guajacol.		
$\frac{1}{1}$ -NaCl	+0,028	
$\frac{1}{1000}$ -NaCl	+0,095	> 0,026
$\frac{1}{100}$ -NaCl	+0,067	> 0,029
$\frac{1}{10}$ -NaCl	+0,038	> 0,015
$\frac{1}{1}$ -NaCl	+0,023	
$\frac{1}{10}$ -HCl	+0,026	> 0,044
$\frac{1}{100}$ -HCl	-0,018	> 0,010
$\frac{1}{1000}$ -HCl	-0,028	
Stearinsäure in Guajacol.		
$\frac{1}{1}$ -KCl	+0,016	
$\frac{1}{100}$ -KCl	+0,039	
$\frac{1}{1000}$ -KCl	+0,070	
$\frac{1}{1}$ -KCl	+0,009	

Wir untersuchten sodann das Verhalten von Ölsäure in anderen Lösungsmitteln und fanden in Orthokresol eine starke Säurewirkung in demselben Sinne, wie wir dieselbe auch bei lebenden Organen beobachtet haben.

TABELLE VIII.

Säurelösung	EMK Volt	Differenz Volt
10% Ölsäure in o-Kresol.		
$\frac{1}{1}$ -KCl	-0,001	
$\frac{1}{10}$ -KCl	+0,010	
$\frac{1}{1200}$ -KCl	+0,065	> 0,011
$\frac{1}{250}$ -KCl	+0,054	> 0,022
$\frac{1}{80}$ -KCl	+0,032	> 0,018
$\frac{1}{10}$ -KCl	+0,014	> 0,013
$\frac{1}{2}$ -KCl	-0,001	
Reines o-Kresol.		
$\frac{1}{10}$ -KCl	-0,006	} schwankend!
$\frac{1}{1000}$ -KCl	+0,001	
$\frac{m}{10}$ -HCl	-0,024	
20% Ölsäure in Amylalkohol.		
$\frac{1}{1}$ -KCl	-0,002	
$\frac{1}{1000}$ -KCl	+0,057	
$\frac{1}{100}$ -KCl	+0,036	
$\frac{1}{10}$ -KCl	+0,006	
$\frac{1}{1}$ -KCl	=0,000	

TABELLE VIII (Fortsetzung).

Säurelösung	EMK Volt	Differenz Volt
Reiner Amylalkohol.		
$\frac{1}{1}$ -KCl	-0,002	
$\frac{1}{1000}$ -KCl	-0,013	
$\frac{1}{100}$ -KCl	-0,012	
$\frac{1}{10}$ -KCl	-0,008	
$\frac{1}{1}$ -KCl	-0,004	

6. Da man sich gewöhnlich vorstellt, dass Lecithin und Cholesterin in der Oberfläche der Zellen auftreten, so untersuchten wir auch das elektromotorische Verhalten von Cholesterin, das in Metakresol gelöst wurde.

TABELLE IX.

10% Cholesterin in Metakresol.

Salzlösung	EMK Volt
$\frac{m}{1}$ -KCl	+0,014
$\frac{m}{10}$ -KCl	+0,017
$\frac{m}{100}$ -KCl	+0,042
$\frac{m}{1000}$ -KCl	+0,042
$\frac{m}{10}$ -KCl	+0,019

Versuche mit Metakresol allein gaben fast dasselbe Verhalten. Cholesterin hat also keinen Einfluss auf das elektromotorische Verhalten.

7. Die nächste Frage war die, ob Fette ebenso wirken wie Phosphatide und wasserunlösliche Fettsäuren. Wir benutzten eine Lösung von 10 Vol.-% Triolein in Guajacol.

TABELLE X.

10% Triolein in Guajacol.

Salzlösung	EMK Volt
$\frac{m}{10}$ -KCl	0,016
$\frac{m}{100}$ -KCl	0,102
$\frac{m}{300}$ -KCl	0,074
$\frac{m}{10}$ -KCl	0,045
$\frac{m}{10}$ -KCl	0,020

Die Säurewirkung fehlt hier ebenso wie bei der freien Ölsäure.

8. Schliesslich machten wir noch Versuche mit Extrakten von Organen in Guajacol, um zu sehen, ob solche Extrakte dasselbe elektromotorische Verhalten zeigen wie die intakten Organe selbst. Apfelschalen wurden erst mit Wasser extrahiert, dann getrocknet und die trockenen Schalen 2 Tage lang mit Guajacol extrahiert. Der Guajacolextrakt wurde dann auf sein elektromotorisches Verhalten geprüft.

TABELLE XI.

Apfelschalensextrakt in Guajacol.

Salzlösung	EMK Volt	Differenz Volt
m-KCl	+0,001	
$\frac{m}{350}$ -KCl	+0,057	
$\frac{m}{1250}$ -KCl	+0,081	
$\frac{m}{350}$ -KCl	+0,058	> 0,023
$\frac{m}{10}$ -KCl	+0,030	> 0,028
$\frac{m}{10}$ -KCl	+0,005	> 0,025
m-KCl	± 0,000	> 0,005

In Bezug auf den Konzentrationseinfluss benimmt sich also der Extrakt der Apfelschale in Guajacol ähnlich wie der Apfel selbst. Der Säureeffekt besteht dagegen nicht.

$$\begin{array}{lcl} \frac{m}{10}\text{-KCl} & . & . & . & + 0,005 \text{ Volt,} \\ \frac{m}{10}\text{-HCl} & . & . & . & + 0,008 \text{ "} \end{array}$$

Es wurde dann ein ähnlicher Versuch mit dem Extrakt von Apfelfruchtfleisch in Guajacol gemacht.

TABELLE XII.

Apfelfruchtfleischextrakt in Guajacol.

Salzlösung	EMK Volt	Differenz Volt
$\frac{1}{1}$ -KCl	-0,006	
$\frac{1}{10}$ -KCl	± 0,000	
$\frac{1}{1250}$ -KCl	+0,088	
$\frac{1}{350}$ -KCl	+0,056	> 0,032
$\frac{1}{10}$ -KCl	+0,019	> 0,037
$\frac{1}{10}$ -KCl	± 0,000	> 0,019
$\frac{1}{1}$ -KCl	-0,006	> 0,006

Hier ist die Annäherung noch besser, vermutlich weil die Extraktion vollständiger war als bei der Rinde des Apfels.

9. Unsere Versuche haben also ergeben, dass sich mit Lösungen von Phosphatiden (Lecithin und Kephalin) in verschiedenen Lösungsmitteln (Guajacol, Metakresol) das elektrische Verhalten der unverletzten Oberfläche lebender pflanzlicher Organe wie des Blattes des Gummibaumes, des Apfels u. a. in allen Einzelheiten nachahmen lässt.

Es lag nahe, daran zu denken, dass die wasserunlöslichen Fettsäuren wie Ölsäure, Stearinsäure, Palmitinsäure, hierfür verantwortlich seien. Es zeigte sich aber, dass das nicht ganz zutrifft, indem die bei lebenden Organen so charakteristische Säurewirkung, die auch bei den Phosphatiden beobachtet wird, bei den Fettsäuren in unseren Versuchen nicht in denselben Lösungsmitteln beobachtet wurde. Cholesterin scheint bei dem Zustandekommen der bioelektrischen Potentialdifferenzen nicht beteiligt zu sein.

ZUSAMMENFASSUNG DER RESULTATE.

1. Es wird gezeigt, dass die bioelektrischen Potentialdifferenzen, die die Verfasser an der Oberfläche unversehrter pflanzlicher Organe beobachtet und früher beschrieben haben, qualitativ und quantitativ nahezu identisch an der Grenze einer Lösung von Phosphatiden (in Guajacol, Metakresol, Amylalkohol) und einer wässrigen Lösung auftreten.

2. Es wird gezeigt, dass ein Extrakt des Apfels in Guajacol sich elektromotorisch genau so benimmt, wie lebende Organe.

3. Dem Guajacol und m-Kresol kommt das hier erwähnte Verhalten teils überhaupt nicht, teils in quantitativ geringem Masse zu, so dass die in 1. und 2. erwähnte Übereinstimmung den Phosphatiden resp. Extraktivstoffen und nicht dem Lösungsmittel zuzuschreiben ist.

4. Die wasserunlöslichen Fettsäuren (Ölsäure, Stearinsäure, Palmitinsäure) zeigen den an lebenden pflanzlichen Organen gefundenen Konzentrationseffekt wässriger Lösungen, dagegen nicht den Säureeffekt. Das gleiche gilt für Triolein. Man kann also das elektromotorische Verhalten der Phosphatide nicht völlig dem Gehalt an Fettsäuren zuschreiben.

5. Cholesterin zeigt kein elektromotorisches Verhalten, das dem lebenden Organe vergleichbar wäre.

6. Feste Eiweisskörper (geronnenes Hühnerei und feste Gelatine) zeigen nicht den Konzentrationseinfluss wässriger Salzlösungen, der nach unseren früheren Arbeiten für die bioelektrischen Potentialdifferenzen charakteristisch ist.

7. Wir dürfen nach allem wohl schliessen, dass die bioelektrischen Potentialdifferenzen dadurch bestimmt sind, dass gewisse lebende pflanzliche Organe an ihrer Oberfläche eine Schicht von Lecithin oder sonstigen Phosphatiden oder ähnlich wirkenden wasserunlöslichen Stoffen besitzen. Variationen in der Natur dieser Stoffe und ihrer Lösungsmittel sind wohl für die quantitativen Verschiedenheiten im elektromotorischen Verhalten verschiedener Organe verantwortlich.

EINFLUSS DER ANAESTHETICA AUF DIE POTENTIAL- DIFFERENZ AN DER OBERFLÄCHE PFLANZ- LICHER UND TIERISCHER GEWEBE.*

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1. In einer früheren Abhandlung¹⁾ haben wir gezeigt, dass die Potentialdifferenz an der unversehrten Oberfläche pflanzlicher Organe und wässriger Salzlösungen scharf definierte und reversible Änderungen mit der Konzentration der Salzlösung aufweist. Der Sinn der Änderung ist derartig, dass von einer Reversibilität für Kationen gesprochen werden kann, die Grössenordnung entspricht nahezu der nach der Nernstschen Formel berechneten. Ein qualitativ ähnliches Verhalten wurde mit verletzten Oberflächen pflanzlicher Organe sowie auch mit tierischen Organen beobachtet.

Wir haben ferner gezeigt, dass Nicht-Elektrolyte, wie Zucker und Harnstoff, keinen Einfluss auf die Potentialdifferenz haben, womit erwiesen ist, dass die von uns beobachteten Erscheinungen nicht durch osmotische Vorgänge hervorgerufen werden. Ganz anders dagegen verhalten sich die Anaesthetica.

2. Einfluss verschiedener Anaesthetica bei konstanter Salzkonzentration. Die Methodik dieser Versuche war ganz die gleiche wie die unserer früheren²⁾. Als Versuchsobjekt diente ein Blatt der Gummipflanze oder ein Apfel; derselbe wurde in eine Schale gelegt, in der sich die Lösung mit dem betreffenden Anaestheticum befand. Eine kleine Menge $\frac{1}{10}$ -KCl-Lösung ohne Zusatz von Anaestheticum wurde auf die obere Seite des unverletzten Apfels aufgebracht und von derselben mit einer $\frac{1}{10}$ -KCl—HgCl-Elektrode zum Messinstrument abgeleitet, die andere ableitende Elektrode (gleicher Art) tauchte in die untere Lösung der

* Eingegangen am 25. April 1913.

¹⁾ Diese Zeitschr. 41, 1, 1912; 44, 303, 1912.

²⁾ Loeb und Beutner, diese Zeitschr. 41, 1, 1912.

Schale. Bei allen Versuchen war eine geringe NaCl-Menge der Lösung in der Schale zugesetzt (meist $\frac{n}{1000}$ -NaCl), denn sonst wäre die Potentialdifferenz in unkontrollierbarer Weise durch die geringsten Salzspuren, die in destilliertem Wasser zugegen sind, beeinflusst worden.

Die tatsächlich gemessene Kette war also:

Kalomel- | Apfel | $\frac{n}{1000}$ -NaCl-Lösung | Kalomel-
Elektrode | mit Zusatz der Anaesthetica | Elektrode.

Für die Narkose ist die Reversibilität entscheidend. Das folgende Beispiel zeigt, bis zu welchem Grade der Einfluss des Anaestheticumzusatzes reversibel ist. Äthylalkohol wurde als Anaestheticum verwendet.

TABELLE I.

Zeit Minuten	Vol.-% absoluter Alkohol in Lösung	EMK Volt
0	0,0	0,119
3	0,0	0,119
6	0,0	0,118
7	2,5	0,114
10	2,5	0,116
19	2,5	0,117
20	5,0	0,117
28	5,0	0,117
30	0,0	0,116
32	10,0	0,110
38	10,0	0,112
46	10,0	0,112
47	20,0	0,099
51	20,0	0,099
52	0,0	0,114
55	40,0	0,081
61	40,0	0,083
70	40,0	0,083
71	0,0	0,116
73	0,0	0,116
74	80,0	0,028
79	80,0	0,030
94	80,0	0,034
95	0,0	0,117
116	0,0	0,120

Diese Zahlen zeigen, dass erst bei dem hohen Alkoholzusatz von $\frac{1}{10}$ des Gesamtvolumens ein deutlicher bleibender Einfluss auf die Potentialdifferenz¹⁾ vorhanden ist. Diese Konzentration ist höher als die sonst zur Narkose verwendeten. Da aber selbst die Wirkung von 80 Vol.-% Alkohol noch reversible Wirkung zeigt, so müssen wir annehmen, dass der Alkohol nur in Spuren in die Rinde des Apfels eindringt. Der Versuch zeigt, dass Alkohol ganz anders wirkt wie Zucker, der ja die Potentialdifferenz ganz unbeeinflusst liess.

3. Weiterhin wurde die Wirkung gleicher Mengen verschiedener Alkohole auf die Potentialdifferenz verglichen. Zu diesen Versuchen konnten nur die 5 Alkohole: Methylalkohol, Äthylalkohol, n-Propylalkohol, Isobutylalkohol und Isoamylalkohol benutzt werden, da alle anderen Alkohole nur begrenzt mischbar mit Wasser sind. Selbst mit Amylalkohol ist die Sättigungskonzentration in Wasser so klein, dass der Einfluss auf die Potentialdifferenz gering ist.

Eine Anzahl von Messungen wurde zunächst mit gleichen Volumprozenten der betreffenden Alkohole ausgeführt. Die hier mitgeteilten Zahlen sind Mittelwerte von Messungen in Intervallen von 3 bis 5 Minuten. Die Reversibilität der Beobachtungen ist in allen Fällen dieselbe wie bei den Äthylalkohol-Wasser-Gemischen.

TABELLE II.

CH ₃ OH			C ₂ H ₅ OH			C ₃ H ₇ OH		
Vol.- %	EMK	Differenz	Vol.- %	EMK	Differenz	Vol.- %	EMK	Differenz
0	0,107		0	0,118		0	0,121	
5	0,110		5	0,117		5	0,122	
0	0,110		0	0,116		10	0,112	
10	0,103	> 0,007	10	0,112	> 0,0004	0	0,122	> 0,010
20	0,097		20	0,099		20	0,104	
0	0,110	> 0,013	0	0,114	> 0,015	0	0,122	> 0,018
40	0,087	> 0,024	40	0,083	> 0,033	40	0,064	> 0,058
0	0,111		0	0,116		0	0,121	
80	0,053		80	0,034				
0	0,112	> 0,059	0	0,117	> 0,083			

¹⁾ Dass in erster Linie die Potentialdifferenz an der Schale des Apfels durch den Alkoholzusatz beeinflusst wird, erscheint wahrscheinlich. Die „Diffusionspotentialdifferenz“ zwischen der Elektrode und der Lösung kann kaum so weitgehend beeinflusst werden.

C_6H_5OH	$C_6H_{11}OH$
$0^0/0 \dots 0,103$ Volt	$0^0/0 \dots 0,123$ Volt
$5^0/0 \dots 0,093$ "	$1^{1/2}0/0 \dots 0,114$ "

(Diese Werte liegen den Sättigungskonzentrationen nahe.)

Vergleichende Messungen mit äquimolekularen Mengen von Methyl-, Äthyl- und Propylalkohol ergaben folgendes Resultat. Die untersuchte Kette war in diesem Falle:

— $^{2/10}$ -Kalomel-Elektrode | Apfel | $^{1/1000}$ n-KCl-Lösung + Anaestheticum | $^{1/10}$ -Kalomel-Elektrode +

TABELLE III.

Erniedrigung der EMK der Kette (in Millivolt).

	Konzentration		
	2,5 n	5,0 n	10,0 n
Zusatz von Methylalkohol.	5 M.V.	7 bis 8 M.V.	28 M.V.
" " Äthylalkohol.	8 bis 11 M.V.	18 bis 22 M.V.	37 M.V.
" " Propylalkohol.	12 M.V.	32 M.V.	104 M.V.

Naturgemäss zeigt sich bei Vergleichung äquimolekularer Lösungen der Einfluss des Molekulargewichts noch stärker wie bei Vergleichung gleicher Volumprocente. Diese Versuche zeigen ferner, dass eine einfache theoretische Beziehung zwischen Äquivalentkonzentration und Änderung der Potentialdifferenz, wie sie für Salzlösungen nachgewiesen werden konnte, hier nicht besteht. Es zeigt sich aber durchaus der negativierende Einfluss des Zusatzes des Anaestheticums.

Es ist auch von Interesse, dass die Wirksamkeit der Alkohole mit der Zahl der Kohlenstoffatome steigt, aber nicht parallel der Narkosewirkung.

4. Versuche mit anderen Anaesthetica gaben ähnliche Resultate. Bei Ätherzusatz war ein deutlicher Einfluss erst nahe der Sättigungskonzentration von 10 Vol.-% zu konstatieren. Die Reversibilität war dann jedoch unvollkommen, wie die folgenden Zahlen zeigen. Gemessen ohne Ätherzusatz

($^{1/10}$ -KCl | Apfel | $^{1/1000}$ -KCl)

128 M.V., darauf 10⁰/0 Vol.-Äther der $^{1/1000}$ -KCl zugefügt, nach

2 Minuten 101 M.V., nach 7 Minuten 93 M.V., darauf ätherfreie $1/1000$ -KCl-Lösung appliziert und wieder gemessen; EMK steigt in 6 Minuten auf 110 M.V. und ist bei diesem Wert noch nach ca. $3/4$ Stunden konstant.

Als nichtflüchtiges Anaestheticum wurde Chloralhydrat untersucht. Der Einfluss auf die Potentialdifferenz erwies sich als sehr gering. Ein Zusatz von 20⁰/₀ Chloral brachte eine Änderung der Potentialdifferenz von nur 5 M.V. (in 22 Minuten) hervor, ein sehr grosser Zusatz von 60⁰/₀, eine solche von 20 M.V. Diese Tatsachen weisen alle darauf hin, dass die Narkotica nur sehr wenig löslich in der Apfelrinde sind.

5. Weitere Versuche betreffen die Frage, ob ein Zusatz von Alkohol in konstanter Konzentration die Veränderlichkeit der Potentialdifferenz mit dem Wechsel der Salzkonzentration beeinflusst. Wie die folgenden Versuche zeigen, besteht ein solcher Einfluss jedoch in nur sehr geringem Grade, die Veränderung der Potentialdifferenz mit der Konzentration der Salzlösung ist kleiner bei Alkoholzusatz. Dies gilt jedoch nur bei Gegenwart des Alkohols in der Lösung. Wiederholt man nachträglich mit alkoholfreier Lösung die Prüfung der Konzentrationsabhängigkeit, so stellen sich in völlig reversibler Weise die ursprünglich beobachteten grösseren Werte wieder ein.

Ohne Alkoholzusatz:

$1/10$ -KCl	10 M.V.	
$1/1000$ -KCl	114 "	> 51 M.V.
$1/100$ -KCl	63 "	> 53 "
$1/10$ -KCl	10 "	

Mit Zusatz von 20 Vol.-⁰/₀ Äthylalkohol:

$1/10$ -KCl	2 M.V.	
$1/1000$ -KCl	93 "	> 47 M.V.
$1/100$ -KCl	46 "	> 44 "
$1/10$ -KCl	2 "	

Mit Zusatz von 50 Vol.-⁰/₀ Äthylalkohol:

$1/1000$ -KCl	64 M.V.	> 33 M.V.
$1/100$ -KCl	31 "	> 33 "
$1/10$ -KCl	—2 "	

6. Frühere Versuche hatten gezeigt¹⁾, dass bei tierischen Objekten der Einfluss der Konzentration der Salze auf die Potentialdifferenz nur gering ist; weitere Versuche am Gastrocnemius des Frosches bestätigen diese Erfahrung; ein Ansteigen der EMK bei Verdünnung war zwar zu bemerken, dasselbe war jedoch durch die Schwankungen der beobachteten Werte fast verdeckt.

Den Einfluss der Anaesthetica beim Muskel zeigt folgender Versuch. Bei Ableitung von Sehne und entgegengesetztem Ende zeigte ein Gastrocnemius die EMK von 6 M.V., wobei letzteres Ende positiv. Bei Zusatz von 10 Vol.-% Alkohol sank diese EMK auf 3 M.V., mit 20 Vol.-% auf — 7 M.V.

Die Wirkung ist also im selben Sinn wie beim Apfel, jedoch etwas geringer.

7. Versuche mit Lecithin. Wir haben in der vorausgehenden Arbeit gezeigt, dass Lecithin, in Guajacol gelöst, dasselbe elektromotorische Verhalten zeigt wie die Oberfläche lebender Organe. Es war daher von Interesse zu untersuchen, ob Zusatz von Alkohol zur wässerigen Lösung die Potentialdifferenz an der Grenze von wässriger Lösung und der Lecithin-Guajacollösung ebenfalls herabsetzt. Es zeigt sich, dass das der Fall ist. Die Versuchsanordnung war dieselbe wie in der vorausgehenden Arbeit.

10 Gewichtsprozent Lecithin
in Guajacol.

$m/_{20}$ -KCl	+ 0,054	Volt
$m/_{20}$ -KCl + $m/_{4}$ -Traubenzucker . . .	+ 0,050	"
$m/_{20}$ -KCl	+ 0,052	"
$m/_{20}$ -KCl in 5 ⁰ /o Alkohol	+ 0,044	"
$m/_{20}$ -KCl " 10 ⁰ /o "	+ 0,034	"
$m/_{20}$ -KCl " 20 ⁰ /o "	+ 0,018	"
$m/_{20}$ -KCl " 50 ⁰ /o "	— 0,001	"

Wir stellten dann noch einen ähnlichen Versuch mit Ölsäure in Guajacol gelöst an. Das Resultat war ebenfalls positiv.

¹⁾ l. c., S. 19.

	10 Vol.-% Ölsäure in Guajacol.
$\frac{m}{20}$ -KCl	+ 0,043 Volt
$\frac{m}{20}$ -KCl in 5 Vol.-% Alkohol . .	+ 0,030 "
$\frac{m}{20}$ -KCl " 10 " "	+ 0,021 "
$\frac{m}{20}$ -KCl " 20 " "	+ 0,009 "
$\frac{m}{20}$ -KCl	+ 0,041 "
$\frac{m}{20}$ -KCl in 50 Vol.-% Alkohol .	— 0,002 "
$\frac{m}{20}$ -KCl " $\frac{m}{4}$ -Traubenzucker . .	+ 0,043 "
$\frac{m}{20}$ -KCl	+ 0,045 "

Wir haben uns auch überzeugt, dass selbst 2 m-Lösungen von Traubenzucker die Potentialdifferenz nicht beeinflussen.

Wir sehen also, dass der Zusatz von Alkohol die Potentialdifferenz an der Grenze von Lecithin oder Ölsäure, in Guajacol gelöst, ebenso herabsetzt, wie an der Grenze von Apfel und wässriger Lösung. Es war ferner nötig, die Veränderung der Potentialdifferenz an reinem Guajacol bei Alkoholzusatz zu prüfen.

	Reines Guajacol.
$\frac{m}{20}$ -KCl	+ 0,018 Volt
$\frac{m}{20}$ -Kl + 50% Alkohol	+ 0,014 "

Die Messung zeigt, dass der Einfluss sehr klein ist; wir sehen also, dass es nur der Gehalt an Ölsäure und Lecithin im Guajacol ist, der die oben beschriebene Wirkung hat.

THEORETISCHE BEMERKUNGEN.

Da Lecithin, Ölsäure, sowie die im lebenden Organ elektromotorisch wirksamen Substanzen wasserunlöslich, aber löslich in Alkohol und Äther sind, so muss die Erklärung für die Herabsetzung der Potentialdifferenz bei Zusatz von Alkohol oder Äther zur wässrigen Phase darauf führen, dass Äther in die wasserunlösliche Phase geht¹⁾.

¹⁾Es sei noch bemerkt, dass, wie Beütner gefunden hat, allgemein der Zusatz von Alkohol die Potentialdifferenz an der Phasengrenze wässriger Lösungen und wasserunmischbarer saurer Stoffe ebenso beeinflusst, wie bei den hier aufgeführten Versuchen. (Zeitschr. f. Elektrochem. 19, 1913.)

ZUSAMMENFASSUNG DER RESULTATE.

1. Es wird gezeigt, dass Zusatz von Alkohol oder Äther zur wässerigen Phase die Potentialdifferenz an der Grenze von lebenden Organen und wässerigen Lösungen herabsetzt. Diese Herabsetzung ist reversibel.
2. Die Mengen von Alkohol und Äther, die diese Wirkung herbeiführen, sind viel grösser als die zur Narkose erforderlichen.
3. Der Zusatz von Äther oder Alkohol zur wässerigen Phase setzt die Potentialdifferenz an der Grenze von Lecithin oder Ölsäure, in Guajacol gelöst, ebenfalls herab.
4. Die Wirkung wird darauf zurückgeführt, dass infolge des Ätherzusatzes etwas Äther in die wasserunlösliche Phase eintritt.
5. Diese Wirkung wird nicht bei Zusatz von indifferenten Nichtleitern, wie etwa Traubenzucker, beobachtet.

THE INFLUENCE OF BASES UPON THE RATE OF OXIDATIONS IN FERTILIZED EGGS.*

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1. In a previous paper we have shown that the weak bases cause a more rapid and greater increase in the rate of oxidations in the *unfertilized* egg than the strong bases.¹ This result differed in principle from the results which Warburg obtained while working on *fertilized* eggs.² He found that NH_4OH does not increase the rate of oxidations in *fertilized* eggs of *Strongylocentrotus lividus* at Naples, while NaOH does; and since NH_4OH diffuses into the egg while NaOH does not, he concludes from this that the effect of OH ions on oxidations is neither determined by their diffusion into the egg nor through their reaction with the plasma membrane, but merely through their presence in the solution surrounding the cell (p. 316). This conclusion does not harmonize with the results which we obtained with the unfertilized egg, in which NH_4OH influenced the oxidations more strongly than the NaOH . We therefore made some experiments on *fertilized* eggs in order to clear up if possible this apparent contradiction. This seemed sufficiently important since it affects not only the theory of fertilization but also the problem of the localization of oxidations in the cell.

Warburg only published the results of three determinations of the rate of oxidations at three different concentrations of NaOH in a mixture of $\text{NaCl} + \text{KCl} + \text{CaCl}_2$ in that proportion in which these salts occur in the sea water. His results were as follows:

* Received for publication, April 18, 1913.

¹ Loeb and Wasteneys: this *Journal*, xiv, p. 355, 1913.

² O. Warburg: *Zeitschr. f. physiol. Chem.*, lxvi, p. 305, 1910.

TABLE I.

Rate of Oxidations in Fertilized Eggs of Strongylocentrotus lividus at Naples.

C _{NaOH}	COEFFICIENT OF OXIDATIONS	
10 ⁻⁸ N	1.4	No segmentation.
10 ⁻⁶ N	3.9	Normal segmentation.
10 ⁻⁴ N	8.1	No segmentation.

It seemed of importance to us to ascertain whether indeed the rate of oxidations in fertilized eggs increased steadily with the increase in the concentration of NaOH. For this reason experiments were carried on with the *fertilized* eggs of *Strongylocentrotus purpuratus* in California. Of freshly fertilized eggs of one female a homogeneous suspension was made and this was divided into five equal lots which were then distributed into five different solutions of NaCl + KCl + CaCl₂ (in the proportion in which these salts exist in the sea water) and various quantities of $\frac{N}{10}$ NaOH were added.

TABLE II.

NATURE OF THE SOLUTION	OXYGEN CONSUMED	COEFFICIENT OF RATE OF OXIDATIONS
	mgm.	
Neutral NaCl + KCl + CaCl ₂	0.44	1.00
50 cc. NaCl + KCl + CaCl ₂ + 0.3 cc. $\frac{N}{10}$ NaOH	0.48	1.08
50 cc. NaCl + KCl + CaCl ₂ + 0.4 cc. $\frac{N}{10}$ NaOH	0.48	1.08
50 cc. NaCl + KCl + CaCl ₂ + 0.5 cc. $\frac{N}{10}$ NaOH	0.50	1.13
50 cc. NaCl + KCl + CaCl ₂ + 0.6 cc. $\frac{N}{10}$ NaOH	0.52	1.18
Neutral NaCl + KCl + CaCl ₂	0.47	1.00
50 cc. NaCl + KCl + CaCl ₂ + 0.7 cc. $\frac{N}{10}$ NaOH	0.55	1.17
50 cc. NaCl + KCl + CaCl ₂ + 0.8 cc. $\frac{N}{10}$ NaOH	0.71(?)	1.51(?)
50 cc. NaCl + KCl + CaCl ₂ + 0.9 cc. $\frac{N}{10}$ NaOH	0.68	1.44
50 cc. NaCl + KCl + CaCl ₂ + 1.0 cc. $\frac{N}{10}$ NaOH	0.73	1.55
Neutral NaCl + KCl + CaCl ₂ (two determinations) {	0.41	
	0.43	
50 cc. NaCl + KCl + CaCl ₂ + 1.1 cc. $\frac{N}{10}$ NaOH	0.76	1.81
50 cc. NaCl + KCl + CaCl ₂ + 1.2 cc. $\frac{N}{10}$ NaOH	0.81	1.93
50 cc. NaCl + KCl + CaCl ₂ + 1.3 cc. $\frac{N}{10}$ NaOH	0.89	2.12
50 cc. NaCl + KCl + CaCl ₂ + 1.4 cc. $\frac{N}{10}$ NaOH	0.79	(eggs injured)

These results seem to indicate one point clearly, namely, that the addition of 0.5 cc. of $\frac{N}{10}$ NaOH or less to 50 cc. of salt solution has

practically no effect upon the rate of oxidations. This experiment was repeated (table III).

The experiment confirms the result of the preceding series. The addition of 0.4 cc. or less of $\frac{N}{10}$ NaOH to 50 cc. of salt solution has no effect upon the rate of oxidations in the fertilized egg, and the addition of 0.8 cc. of $\frac{N}{10}$ NaOH has only a very slight effect.

TABLE III.

NATURE OF THE SOLUTION	OXYGEN CONSUMED	COEFFICIENT OF RATE OF OXIDATIONS
	<i>mgm.</i>	
Neutral NaCl + KCl + CaCl ₂	0.58	1.00
50 cc. NaCl + KCl + CaCl ₂ + 0.1 cc. $\frac{N}{10}$ NaOH	0.58	1.00
50 cc. NaCl + KCl + CaCl ₂ + 0.2 cc. $\frac{N}{10}$ NaOH	0.53	0.90
50 cc. NaCl + KCl + CaCl ₂ + 0.4 cc. $\frac{N}{10}$ NaOH	0.60	1.00
50 cc. NaCl + KCl + CaCl ₂ + 0.8 cc. $\frac{N}{10}$ NaOH	0.83	1.40

We next tried the effect of another strong base, namely, tetraethylammoniumhydroxide, on the rate of oxidations in fertilized eggs of *S. purpuratus*. The following table gives the result.

TABLE IV.

NATURE OF THE SOLUTION	OXYGEN CONSUMED	COEFFICIENT OF RATE OF OXIDATIONS
	<i>mgm.</i>	
Neutral NaCl + KCl + CaCl ₂	0.45	1.00
50 cc. NaCl + KCl + CaCl ₂ + 0.2 cc. $\frac{N}{10}$ N(C ₂ H ₅) ₄ OH	0.44	1.00
50 cc. NaCl + KCl + CaCl ₂ + 0.4 cc. $\frac{N}{10}$ N(C ₂ H ₅) ₄ OH	0.44	1.00
50 cc. NaCl + KCl + CaCl ₂ + 0.5 cc. $\frac{N}{10}$ N(C ₂ H ₅) ₄ OH	0.47	1.00

The addition of 0.5 cc. of $\frac{N}{10}$ N(C₂H₅)₄OH to 50 cc. of salt solution does not influence the rate of oxidation in the fertilized eggs of *S. purpuratus*. The addition of more N(C₂H₅)₄OH would interfere too much with the analytical result and was not undertaken.

2. Weak bases influence the rate of oxidations in the fertilized egg of *Strongylocentrotus purpuratus* but slightly. This is shown in table V for NH₄OH and in table VI for methylamine.

This result agrees with the observation of Warburg.

3. We have to explain two groups of facts:

1. The apparent difference in the action of weak and strong bases on fertilized eggs.

2. The difference in the reaction of fertilized and unfertilized eggs. We will take up these questions in succession. The fact that

TABLE V.

NATURE OF THE SOLUTION	OXYGEN CONSUMED	COEFFICIENT OF RATE OF OXIDATIONS
	mgm.	
Neutral NaCl + KCl + CaCl ₂ { <i>a</i>	0.43	
..... { <i>b</i>	0.47	
50 cc. NaCl + KCl + CaCl ₂ + 0.6 cc. $\frac{N}{10}$ NH ₄ OH.....	0.66	1.47
50 cc. NaCl + KCl + CaCl ₂ + 1.0 cc. $\frac{N}{10}$ NH ₄ OH.....	0.65	1.44
50 cc. NaCl + KCl + CaCl ₂ + 1.4 cc. $\frac{N}{10}$ NH ₄ OH.....	0.61	1.36
50 cc. NaCl + KCl + CaCl ₂ + 1.6 cc. $\frac{N}{10}$ NH ₄ OH.....	0.59	1.31
50 cc. NaCl + KCl + CaCl ₂ + 1.8 cc. $\frac{N}{10}$ NH ₄ OH.....	0.63	1.40
50 cc. NaCl + KCl + CaCl ₂ + 2.0 cc. $\frac{N}{10}$ NH ₄ OH.....	0.61	1.36
Neutral NaCl + KCl + CaCl ₂	0.55	
50 cc. NaCl + KCl + CaCl ₂ + 0.1 cc. $\frac{N}{10}$ NH ₄ OH.....	0.60	1.09
50 cc. NaCl + KCl + CaCl ₂ + 0.3 cc. $\frac{N}{10}$ NH ₄ OH.....	0.74	1.35
50 cc. NaCl + KCl + CaCl ₂ + 0.6 cc. $\frac{N}{10}$ NH ₄ OH.....	0.69	1.25
50 cc. NaCl + KCl + CaCl ₂ + 1.0 cc. $\frac{N}{10}$ NH ₄ OH.....	0.74	1.35
50 cc. NaCl + KCl + CaCl ₂ + 1.8 cc. $\frac{N}{10}$ NH ₄ OH.....	0.70	1.27
50 cc. NaCl + KCl + CaCl ₂ + 2.4 cc. $\frac{N}{10}$ NH ₄ OH.....	0.72	1.31
50 cc. NaCl + KCl + CaCl ₂ + 3.0 cc. $\frac{N}{10}$ NH ₄ OH.....	0.73	1.33

TABLE VI.

NATURE OF THE SOLUTION	OXYGEN CONSUMED	
	mgm.	
Neutral NaCl + KCl + CaCl ₂	0.41	
50 cc. NaCl + KCl + CaCl ₂ + 0.4 cc. $\frac{N}{10}$ methylamine....	0.46	
50 cc. NaCl + KCl + CaCl ₂ + 0.6 cc. $\frac{N}{10}$ methylamine....	0.44	
50 cc. NaCl + KCl + CaCl ₂ + 0.8 cc. $\frac{N}{10}$ methylamine....	0.40	
Neutral NaCl + KCl + CaCl ₂	0.42	
50 cc. NaCl + KCl + CaCl ₂ + 1.0 cc. $\frac{N}{10}$ methylamine....	0.34	
50 cc. NaCl + KCl + CaCl ₂ + 1.2 cc. $\frac{N}{10}$ methylamine....	0.27	} eggs have suffered considerably and increasingly.
50 cc. NaCl + KCl + CaCl ₂ + 1.4 cc. $\frac{N}{10}$ methylamine....	0.26	

weak bases have no effect upon the rate of oxidations in fertilized eggs of *purpuratus* finds its explanation in the fact that strong bases do not act either except if their concentration is above $10^{-3}N$. If we assume that this effect is due to the concentration of OH ions it would require a concentration of $\frac{x}{1000} NH_4OH$ or more to produce a similar effect, since the degree of dissociation of NH_4OH is so much lower than that of $NaOH$. Such a concentration would probably destroy the eggs very rapidly.

It seems very important to point out that we cannot draw any conclusion from this experiment upon the seat of normal oxidations in the egg, since that concentration of $NaOH$ required to raise the rate of oxidations in the fertilized egg is injurious. We ascertained the maximum concentration of $NaOH$ which allows the egg to develop normally (in a mixture of $NaCl + KCl + CaCl_2$) and found that it varies somewhat between 0.2 and 0.4 cc. of $\frac{x}{100} NaOH$ to 50 cc. of solution. The minimal concentration in which $NaOH$ increases the rate of oxidations in the fertilized egg lies above this limit. We are therefore dealing with a kind of injurious (etching?) effect of the $NaOH$, which is probably in no way related to the normal processes of oxidations. The weaker bases do not produce this injurious (etching?) effect in the concentrations in which they can be applied and this accounts for the fact that in the fertilized eggs an increase in the rate of oxidations can apparently only be produced by strong and not by weak bases.

The second, and perhaps more interesting question, is, why the weak bases influence the rate of oxidations in the unfertilized eggs so powerfully while they are absolutely ineffective in the fertilized egg. There are two possible explanations. First, it may be said that the bases raise the rate of oxidations in the unfertilized egg only indirectly by causing a liquefaction or cytolysis of the cortical layer. This in itself causes, as was shown by Warburg and confirmed by ourselves, a rise in the rate of oxidations, no matter by which means this cytolysis was caused.

It is secondly possible that the cytolysis of the cortical layer by bases is the result of oxidation, since the bases do not induce development in the unfertilized egg if the oxidations are suppressed. If this be true we must assume that the cortical layer contains a sub-

stance which can be liquefied by bases directly or indirectly through oxidations, and more rapidly by the weak bases than by the strong ones, since the weak bases diffuse more rapidly into the egg. Since the spermatozoon liquefies this substance also it becomes clear that the rate of oxidations in the fertilized egg is no longer affected by weak bases.

SUMMARY OF RESULTS.

1. It is shown that bases influence the rate of oxidations differently in the fertilized and unfertilized egg of *Strongylocentrotus purpuratus*.

2. Strong bases, like NaOH and $N(C_2H_5)_4OH$, accelerate the rate of oxidations in the fertilized egg of *S. purpuratus* only if their concentration is above $10^{-3}N$; this concentration suppresses the phenomena of development of the egg.

3. Weak bases, like NH_4OH and methylamine, accelerate the rate of oxidations in the fertilized egg only slightly; the reason being that the weak bases are too slightly dissociated to produce in the concentrations admissible in the experiment the injurious (etching?) effects which the highly dissociated bases produce in this case.

4. These facts do not warrant any conclusion concerning the localization of the normal processes of oxidations in the egg.

THE INFLUENCE OF HYPERTONIC SOLUTION UPON THE RATE OF OXIDATIONS IN FERTILIZED AND UNFERTILIZED EGGS.*

By JACQUES LOEB AND HARDOLPH WASTENEYS.

(From the Laboratories of The Rockefeller Institute for Medical Research,
New York.)

I.

In a series of papers published since 1905 one of the writers has shown that in the egg of the sea urchin the initiation of normal development requires two different agencies. The one is needed to call forth a typical change in the surface of the egg which results in the formation of a more or less typical "fertilization membrane." This change causes the egg to segment and if the temperature is low some of these eggs may reach an early larval stage. At room temperature, however, the eggs begin to disintegrate after artificial membrane formation, as a rule, during the first cell division. These facts prove that the artificial membrane formation (*e. g.*, by butyric acid) suffices to set the whole machinery of cell division and development into motion but that the egg is sickly and disintegrates the more rapidly the higher the temperature.¹

It was shown by the same author that this disintegration after artificial membrane formation is retarded or suppressed if we deprive the egg of oxygen or if we retard the rate of oxidations in the egg through the addition of a trace of KCN. The addition of a sufficient amount of chloral hydrate (or probably any other narcotic) acts in the same way, although chloral hydrate does not lower the rate of oxidations. But all these agencies have one object in common, namely, that they inhibit the processes of nuclear

* Received for publication, April 21, 1913.

¹ Loeb: *Die chemische Entwicklungserregung des tierischen Eies*, Berlin, 1909;
The Mechanistic Conception of Life, 1912.

and cell division. The disintegration of the unfertilized egg after membrane formation is therefore connected either with oxidations or with the nuclear and cell divisions consequent upon oxidations. We can cure the egg from this disease by one of two agencies: We either put it for a short time (from 30 to 60 minutes) into a neutral hypertonic solution; or we put it for a longer time (about 3 hours) into sea water which is free from oxygen or which contains some KCN. The former method gives more uniform results. Such eggs develop into larvae at room temperature.

These results gained in importance since it could be shown that the developmental effect of the spermatozoon is also due to two different agencies, one of which causes merely membrane formation while the other produces the corrective effect.²

Warburg had already found that the artificial membrane formation raises the rate of oxidations in the egg to the same height as the entrance of the spermatozoon, as was to be expected and as we were able to confirm.³ Since Loeb had found that the hypertonic solution acts only in the presence of free oxygen and that its action is suppressed by the addition of a trace of KCN, it was of interest to find out whether or not the hypertonic solution alters the rate of oxidations in the egg after artificial membrane formation. The experiments were made in this way, that the rate of oxidations in the eggs, after artificial membrane formation, was determined, first in normal sea water and later in hypertonic solution.

These experiments are by no means simple, since it is necessary that all the eggs possess membranes; for we shall see afterwards that in unfertilized eggs without membranes the hypertonic solution causes a decided rise in the rate of oxidations.

The unfertilized eggs of one female, *Strongylocentrotus purpuratus*, were divided into six equal parts. Two remained unaltered and served as checks. The eggs of two lots were treated with butyric acid and all formed membranes. One of these lots was put into normal sea water, the other into hypertonic sea water (50 cc. sea water + 8 cc. $\frac{6M}{2}$ NaCl) such as is used to cause the

² Loeb: *loc. cit.*

³ Warburg: *Zeitschr. f. physiol. Chem.*, lxvi, p. 305, 1910.

eggs to develop normally after artificial membrane formation. The fifth lot was fertilized with sperm. The sixth lot served for some other experiment which does not concern us here. The eggs remained in each solution one hour. Temperature 18° C.

TABLE I.

	OXYGEN CONSUMED	COEFFICIENT OF OXIDATIONS
	<i>mgm.</i>	
1. Unfertilized eggs in normal sea water (control 1)	0.12	1.00
2. Unfertilized eggs in normal sea water (control 2)	0.12	1.00
3. Unfertilized eggs after membrane formation in normal sea water	0.53	4.40
4. Unfertilized eggs after membrane formation in hypertonic sea water	0.54	4.50
5. Fertilized eggs in normal sea water	0.57	4.70

It is obvious that the hypertonic solution does not increase the rate of oxidations in the unfertilized eggs after artificial membrane formation. Table II gives the result of a second experiment of the same kind. Temperature 15° C.

TABLE II.

	OXYGEN CONSUMED PER HOUR	COEFFICIENT OF OXIDATIONS
	<i>mgm.</i>	
1. Unfertilized eggs in normal sea water	0.18	1.00
2. Unfertilized eggs after membrane formation in normal sea water	0.85	4.72
3. Unfertilized eggs after membrane formation in hypertonic sea water	0.88	4.88
4. Fertilized eggs in normal sea water	0.82	4.55

The result is identical with that in table I. Membrane formation raises the rate of oxidations to the same height as fertilization, but the subsequent treatment of these eggs with the hypertonic solution has no effect upon the rate of oxidations. This experiment was repeated three times with the same results as shown in Table III. The oxygen consumption is always for one hour at 18° C.

All these experiments prove conclusively that the curative effect of the hypertonic solution after the artificial membrane formation is not due to an increase in the rate of oxidations in the egg. It

cannot be said, however, that it is independent of oxidations, since the curative effect of the hypertonic solution is retarded or suppressed if the oxidations in the egg are suppressed. Loeb formerly suggested that through the exposure to the hypertonic solution an oxidation product is formed in the egg whereby the latter is saved

TABLE III.

NUMBER OF EXPERIMENT		OXYGEN CONSUMED PER HOUR	COEFFICIENT OF OXIDATIONS
		<i>mgm.</i>	
1	Unfertilized eggs after membrane formation in normal sea water.	0.83	1.00
	Unfertilized eggs after membrane formation in hypertonic sea water.	0.74	0.90
2	Unfertilized eggs after membrane formation in normal sea water.	0.52	1.00
	Unfertilized eggs after membrane formation in hypertonic sea water.	0.54	1.04
3	Unfertilized eggs after membrane formation in normal sea water.	0.74	1.00
	Unfertilized eggs after membrane formation in hypertonic sea water.	0.70	0.90

from the threatening disintegration. It may be that an injurious substance contained in the egg after membrane formation is destroyed or that a new substance lacking in the egg is supplied. The same curative effect can be produced more slowly through other processes in the egg which take place in the absence of oxygen.

II. THE INFLUENCE OF HYPERTONIC SOLUTIONS UPON THE RATE OF OXIDATIONS IN FERTILIZED EGGS.

Since the unfertilized egg after artificial membrane formation behaves in regard to oxidations like a fertilized egg, it was of interest to find out whether or not the hypertonic solution accelerates the rate of oxidations in eggs fertilized by sperm. O. Warburg states that this is the case, and that the increase may be 300 per cent.⁴ We absolutely failed to notice any increase in the rate of oxidations when the eggs of *S. purpuratus* were put into hypertonic sea water, no matter how great the degree of hypertonicity. In part of the experiments, 1-3, the rate of oxidations was successively

⁴ Warburg: *Zeitschr. f. physiol. Chem.*, 1x, p. 442, 1909.

measured in the same eggs for one and a half hours in normal sea water, and then for one hour in the hypertonic sea water. In the experiments 4-7 the eggs of the same female were divided into eight equal parts; four of these were put into normal sea water, the others into hypertonic sea water of different concentrations. This experiment may incidentally also serve as a check for the accuracy of the method.

The temperature was 18° C. and the consumption of oxygen measured for one and a half hours. Table IV gives the results.

There can be no doubt about the fact that the hypertonic solu-

TABLE IV.

NUMBER OF EXPERIMENT		OXYGEN CONSUMED	COEFFICIENT OF RATE OF OXIDATIONS
		<i>mgm.</i>	
1	Fertilized eggs in normal sea water.....	0.87	1.00
	Fertilized eggs in 50 cc. normal sea water + 8 cc. $\frac{5M}{2}$ NaCl.....	0.86	0.99
2	Fertilized eggs in normal sea water.....	0.60	1.00
	Fertilized eggs in 50 cc. normal sea water + 8 cc. $\frac{5M}{2}$ NaCl.....	0.52	0.87
3	Fertilized eggs in normal sea water.....	0.55	1.00
	Fertilized eggs in 50 cc. normal sea water + 8 cc. $\frac{5M}{2}$ NaCl.....	0.59	1.07
4	Fertilized eggs in normal sea water.....	1.30	1.00
	Fertilized eggs in 50 cc. normal sea water + 4 cc. $\frac{5M}{2}$ NaCl.....	1.27	0.98
5	Fertilized eggs in normal sea water.....	1.30	1.00
	Fertilized eggs in 50 cc. normal sea water + 12 cc. $\frac{5M}{2}$ NaCl.....	1.54	1.20
6	Fertilized eggs in normal sea water.....	1.33	1.00
	Fertilized eggs in 50 cc. normal sea water + 12 cc. $\frac{5M}{2}$ NaCl.....	1.53	1.20
7	Fertilized eggs in normal sea water.....	1.33	1.00
	Fertilized eggs in 50 cc. normal sea water + 16 cc. $\frac{5M}{2}$ NaCl.....	0.57	1.20

tion does not increase the rate of oxidations in the fertilized egg of *Strongylocentrotus purpuratus*. This harmonizes with our previous result that the hypertonic solution does not increase the rate of oxidations in unfertilized eggs of the same species after artificial

membrane formation, since the membrane formation is the essential feature in the causation of development by sperm or by artificial means.

III.

The fact that a hypertonic solution does not increase the rate of oxidations in the unfertilized eggs after artificial membrane formation seems at first sight to contradict an observation made by Warburg at Naples, that the hypertonic solution raised the rate of oxidations in unfertilized eggs (which have not been submitted to the process of membrane formation). Warburg found that the hypertonic solution raises the rate of oxidations in such eggs as much as ten times.⁵ We repeated these experiments on the eggs of *Strongylocentrotus purpuratus* at Pacific Grove, and were able to confirm Warburg's results although the rise in the rate of oxidations was much smaller than that observed in his experiments. In our experiments the rate of oxidations was first determined for one and a half hours in normal sea water and then for one and a half hours in hypertonic sea water. Table V on the following page gives the results. The temperature was 18° C.

It should be pointed out that experiments 2 and 3, and 4 and 5, are made on equal lots of eggs. The results may serve as a check for the accuracy of the method.

The question arises: Why is it that the hypertonic solution causes a rise in the rate of oxidations in the unfertilized egg without membrane formation, while it has no such effect on the same eggs after membrane formation? The answer is that the hypertonic solution can cause the membrane formation and that it only raises the rate of oxidations in those eggs in which it causes membrane formation. In Loeb's original method of causing artificial parthenogenesis by merely putting the eggs into a hypertonic solution, the hypertonic solution had two kinds of effects: it caused first the membrane formation (or the formation of a gelatinous surface film) and at the same time furnished the curative effect.

That the hypertonic solution can cause a cytolytic effect upon the cortical layer of the egg was shown by experiments on the eggs

⁵ Warburg: *Zeitschr. f. physiol. Chem.*, lx, p. 443, 1909.

TABLE V.

NUMBER OF EXPERIMENT	UNFERTILIZED EGGS WITHOUT MEMBRANES IN	OXYGEN CONSUMED	COEFFICIENT OF RATE OF OXIDATIONS
		<i>mgm.</i>	
1	Normal sea water.....	0.45	1.00
	Hypertonic (50 cc. sea water + 4 cc. $\frac{5M}{2}$ NaCl)	0.65	1.40
2	Normal sea water.....	0.50	1.00
	Hypertonic (50 cc. sea water + 6 cc. $\frac{5M}{2}$ NaCl)	0.92	1.80
3	Normal sea water.....	0.49	1.00
	Hypertonic (50 cc. sea water + 6 cc. $\frac{5M}{2}$ NaCl)	0.84	1.70
4	Normal sea water.....	0.33	1.00
	Hypertonic (50 cc. sea water + 6 cc. $\frac{5M}{2}$ NaCl)	0.73	2.20
5	Normal sea water.....	0.35	1.00
	Hypertonic (50 cc. sea water + 8 cc. $\frac{5M}{2}$ NaCl)	0.73	2.50
6	Normal sea water.....	0.46	1.00
	Hypertonic (50 cc. sea water + 8 cc. $\frac{5M}{2}$ NaCl)	1.19	2.60
7	Normal sea water.....	0.48	1.00
	Hypertonic (50 cc. sea water + 9 cc. $\frac{5M}{2}$ NaCl)	1.23	2.60
8	Normal sea water.....	0.29	1.00
	Hypertonic (50 cc. sea water + 9 cc. $\frac{5M}{2}$ NaCl)	0.90	3.10
9	Normal sea water.....	0.30	1.00
	Hypertonic (50 cc. sea water + 9 cc. $\frac{5M}{2}$ NaCl)	0.67	2.20
10	Normal sea water.....	0.56	1.00
	Hypertonic (50 cc. sea water + 12 cc. $\frac{5M}{2}$ NaCl)	1.29	2.30
11	Normal sea water.....	0.38	1.00
	Hypertonic (50 cc. sea water + 16 cc. $\frac{5M}{2}$ NaCl)	0.97	2.60

of *Lottia* (a mollusc) whose chorion can be liquefied by hypertonic solutions as well as by bases, if free oxygen is present.⁶

That the two agencies, the membrane-forming and the corrective one, can act simultaneously is not surprising. The same fact can be shown for bases. It is immaterial whether the base is applied first and is then followed by the corrective action of the hypertonic solution or whether both agencies are applied simultaneously.

In our experiments the effect of the hypertonic solution upon oxidations was smaller than that caused by membrane formation with butyric acid. In the latter case, the rate of oxidations was raised from four to six times, while the hypertonic solution, when acting upon unfertilized eggs without membrane formation, raised

⁶ Loeb: *Univ. of Calif. Publ. Physiol.*, iii, 1905.

the rate as a rule to not more than two and one-half and at the utmost three times the amount observed in the same eggs in normal sea water. This difference can be accounted for either by the fact that an exposure of one and a half hours of the eggs of *S. purpuratus* to hypertonic sea water as a rule does not suffice to bring about membrane formation and development; or by the fact that these experiments were made early in the season when fertilization did not raise the rate of oxidations as much as it did later in the season.

We repeated these experiments later in the season to find out whether the rate of oxidations would increase if the eggs remained a longer time in the hypertonic sea water. The temperature was 18° C. The consumption of oxygen was determined for each hour.

TABLE VI.

UNFERTILIZED EGGS IN	OXYGEN CONSUMED	COEFFICIENT OF RATE OF OXIDATIONS
	<i>mgm.</i>	
Normal sea water	0.16	1.00
Hypertonic sea water; 1st hour	0.67	4.18
Hypertonic sea water; 2d hour	0.79	4.94
Hypertonic sea water; 3d hour	0.64	4.00
Hypertonic sea water; 4th hour	0.56	3.55
Hypertonic sea water; 5th hour	0.51	3.18

A repetition of the experiment gave a similar result. In this case the hypertonic solution had the same effect upon the rate of oxidations as fertilization or membrane formation. It may also be that by chance we are dealing in this case with the eggs of a female which were very susceptible to the treatment with hypertonic solution while in the previous experiments the eggs had been more or less refractory. As we stated, not the eggs of every female of *purpuratus* develop if treated with hypertonic sea water.

These results support also the idea that the rise in the rate of oxidations is due to the membrane-forming action of the hypertonic solution, which of course takes place during the first two hours. We see that the maximal increase in the rate of oxidations takes place during that time.

IV.

If it is true that the increase in the rate of oxidations observed in these cases is merely due to the membrane-forming effect of the hypertonic solution, we should expect that if we add a weak base to the hypertonic solution, the increase in the rate of oxidations should be no greater than that caused by the weak base alone, the reason being that the weak base alone causes the membrane formation.⁷ This reasoning is supported by facts. The rate of oxidations was compared in unfertilized eggs (without membranes) in alkaline sea water and hypertonic sea water to which the same amount of base had been added. The hypertonic sea water consisted of 50 cc. sea water + 8 cc. $\frac{5M}{2}$ NaCl + KCl + CaCl₂. The eggs were distributed into equal portions. One-half was put into 50 cc. of sea water + 1 cc. of the base; the other half of the eggs was first put into normal sea water and then into 50 cc. of hypertonic sea water + 1 cc. of the same base. Time of exposure one and a half hours; temperature 18° C.

TABLE VII.

NUMBER OF EXPERIMENT	UNFERTILIZED EGGS (WITHOUT MEM- BRANES) IN	OXYGEN CONSUMED	COEFFICIENT OF RATE OF OXIDATIONS
		mgm.	
1	Normal sea water.....	0.22	1.00
	50 cc. hypertonic sea water + 1 cc. $\frac{N}{10}$ NH ₄ OH	1.20	5.40
2	50 cc. normal sea water + 1 cc. $\frac{N}{10}$ NH ₄ OH..	0.88	4.00
	Normal sea water.....	0.37	1.00
3	50 cc. hypertonic sea water + 1 cc. $\frac{N}{10}$ benzyl- amine.....	1.89	5.10
	50 cc. normal sea water + 1 cc. $\frac{N}{10}$ benzyl- amine.....	1.75	4.70
3	Normal sea water.....	0.36	1.00
	50 cc. hypertonic sea water + 1 cc. $\frac{N}{10}$ butyl- amine.....	1.73	4.80
	50 cc. normal sea water + 1 cc. $\frac{N}{10}$ butyl- amine.....	1.67	4.60

It is obvious that the weak base alone raised the rate of oxidations practically to the same height as the combination of base and hypertonic sea water. The whole rise was due to the membrane-forming effect for which the weak base was sufficient.

⁷ Loeb: *Journ. of Exp. Zoölogy*, xiii, p. 577, 1912.

In the case of a strong base, the result may be different, since neither the base nor the hypertonic solution alone may cause membrane formation (or the change in the cortical layer of the egg) necessary for development. The following may serve as an example. Duration of experiment one and a half hours; temperature 18° C.

TABLE VIII.

UNFERTILIZED EGGS (WITHOUT MEMBRANE FORMATION) IN	OXYGEN CONSUMED	COEFFICIENT OF RATE OF OXIDATIONS
	<i>mgm.</i>	
Normal sea water	0.41	1.00
50 cc. hypertonic sea water + 1 cc. $\frac{N}{10}$ NaOH	0.81	2.00
50 cc. normal sea water + 1 cc. $\frac{N}{10}$ NaOH	0.46	1.20

In this case the NaOH had little effect and hence the hypertonicity caused a noticeable increase in the rate since it probably increased the number of eggs in which the process of membrane formation was started.

V.

Finally, we wish to report an experiment which does not strictly belong here but which shows that the mere cytolysis of the cortical layer is responsible for the increase in the rate of oxidations observed after membrane formation either by a spermatozoon or by butyric acid. It is possible to cause complete cytolysis of the unfertilized egg of *S. purpuratus* with saponin and we found that this increases the rate of oxidations to the same extent as fertilization by sperm. The following may serve as an example. Unfertilized eggs of *S. purpuratus* were used; temperature 15° C.

TABLE IX.

	OXYGEN CONSUMED PER HOUR	COEFFICIENT OF RATE OF OXIDATIONS
	<i>mgm.</i>	
Unfertilized eggs	0.15	1.00
The same eggs after cytolysis with saponin	1.07	7.10
Unfertilized eggs	0.22	1.00
The same eggs after cytolysis with saponin	0.80	3.60

The variation in the effect of cytolysis in the two experiments may be due to the fact that in the second experiment an excessive amount of saponin was used.

This experiment proves that the increase in the rate of oxidations due to fertilization or artificial membrane formation is merely caused by the cytolysis of the cortical layer.

VI. THEORETICAL REMARKS.

It seems that all the experiments point very clearly towards one conclusion, namely, that the hypertonic solution raises the rate of oxidations in the unfertilized eggs of *S. purpuratus* only under one condition, *i.e.*, if it causes the change at the surface of the egg underlying membrane formation. In eggs which have undergone the process of membrane formation either by fertilization or by a treatment with butyric acid, the hypertonic solution causes no further increase in the rate of oxidations.

SUMMARY.

1. The unfertilized eggs of sea urchins which have undergone artificial membrane formation die if not treated with a hypertonic solution. It is shown in these experiments that the rate of oxidations in such eggs is not increased by the hypertonic solution.

2. It is shown that the hypertonic solution does not cause an increase in the rate of oxidations of fertilized eggs of *Strongylocentrotus purpuratus*.

3. Hypertonic solutions increase the rate of oxidations in unfertilized eggs which have not undergone the process of membrane formation, as Warburg observed. This increase is purely due to the fact that the hypertonic solution induces the change in the cortical layer of the egg which leads to membrane formation.

4. This conclusion is supported by the fact shown in this paper, that the addition of a weak base to normal sea water, which causes development (or membrane formation), increases the rate of oxidations in unfertilized eggs to the same amount as if it were added to hypertonic sea water. Since in this case the membrane-forming effect can be produced by the base alone the addition of the hypertonic solution can add nothing to the effect.

5. Complete cytolysis of the unfertilized egg by saponin raises the rate of oxidations to the same height as fertilization, thus showing that the cytolysis of the cortical layer of the egg is the essential feature in fertilization.

IS NARCOSIS DUE TO ASPHYXIATION?*

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New York.)

1. The idea that the phenomenon of narcosis might be caused by an interference with oxidation is an old one. Recently it has been advocated by Verworn who tried to support it by indirect evidence.¹ Yet it is obvious that if we wish to ascertain whether or not narcosis is due to a diminution in the rate of oxidations (or, as Verworn expresses it, to an asphyxiation) there is only one way to decide the question, namely by comparing the rate of oxidations in narcotized organisms with that in non-narcotized.

If we wish to carry out such experiments we must keep in mind that organisms which are capable of muscular action cannot well be used for this purpose since we know that muscular activity can easily raise the rate of oxidation in the animal body one hundred per cent. Since the narcotics cause muscular action to cease, a narcotized animal will consume less oxygen than a free-moving animal; but it would be a mistake to state that this diminution in the consumption of oxygen was the cause of narcosis. It is the effect and not the cause of narcosis. If we wish to test the asphyxiation hypothesis of narcosis we must use organisms which possess no muscles and in which therefore this source of error does not interfere with the result. The best material for this purpose is offered in the fertilized eggs. Loeb had shown in 1895 that the eggs of the sea urchin and fish cannot develop if the oxygen is withdrawn² and this result has since been extended to a number of eggs of different forms. The same author showed that the addition of a small amount

* Received for publication, April 30, 1913.

¹ Verworn: *The Harvey Lectures*, 1911-12, p. 52.

² Loeb: *Pflüger's Archiv*, lxii, p. 249, 1895.

of KCN also inhibits segmentation. We shall see later that KCN only has this effect if it depresses the rate of oxidations in the egg below a certain level. The suppression of segmentation by lack of oxygen as well as by KCN is a reversible process.

It is also well known that anaesthetics inhibit the segmentation of the fertilized sea urchin egg reversibly. It was, therefore, an easy task to compare the rate of oxidations in equal quantities of fertilized eggs with and without the presence of narcotics. Warburg had already observed that phenyl urethane in the concentration in which it suppressed nuclear and cell division did not diminish the rate of oxidations. Only if an excessive amount of phenyl urethane was added, was a diminution in the rate of oxidations noticeable; but, as he correctly adds, the question is not whether it is at all possible to lower the rate of oxidations by adding narcotics but whether that concentration which suffices for narcosis lowers the rate of oxidations.³

We undertook a series of experiments on the effect of various narcotics upon the rate of oxidations in the newly fertilized eggs of *Strongylocentrotus purpuratus*. Since the method of procedure and the degree of reliability of our method have been described in previous papers no recapitulation of these points is needed.

2. *Experiments with KCN.*—We determined the minimum amount of KCN necessary to suppress cell division permanently in the newly fertilized eggs of *S. purpuratus* and found that the addition of 0.7 cc. of 0.01 per cent KCN to 50 cc. of sea water was the minimum amount required. We then determined the influence of various amounts of KCN upon the rate of oxidations. Newly fertilized eggs were divided into equal lots and the amount of oxygen consumed in one hour was determined. Temperature 14° C.

TABLE I.

	OXYGEN CONSUMED	RATE OF OXIDATIONS
	<i>mgm.</i>	
1. Normal sea water	0.51	1.00
2. 50 cc. sea water + 0.7 cc. 0.01 per cent KCN	0.17	0.33
3. 50 cc. sea water + 0.9 cc. 0.01 per cent KCN	0.15	0.29

³ O. Warburg: *Zeitschr. f. physiol. Chem.*, lxvi, p. 305, 1910.

In solution 1, the eggs segmented; in solutions 2 and 3 no segmentation occurred. The eggs were transferred from solutions 2 and 3 to normal sea water and all developed into normal larvae. The amount of KCN necessary to suppress cell division lowers the rate of oxidations to one-third of the normal amount. A repetition of the experiment confirmed the result.

TABLE II.

	OXYGEN CONSUMED	RATE OF OXIDATIONS
	<i>mgm.</i>	
1. Normal sea water.....	0.65	1.00
2. 50 cc. sea water + 0.4 cc. 0.01 per cent KCN.....	0.34	0.52
3. 50 cc. sea water + 0.7 cc. 0.01 per cent KCN.....	0.21	0.32
4. 50 cc. sea water + 1.0 cc. 0.01 per cent KCN....	0.18	0.28

The addition of 0.4 cc. of KCN only retarded the development but did not suppress it. The addition of 0.7 cc. of KCN suppressed cell division, and the rate of oxidations was again exactly one-third of the normal.

Similar results had previously been obtained by us for the eggs of *Arbacia*.⁴

We can, therefore, state that if the rate of oxidations of the egg is lowered to one-third of the normal amount found at 14° C., the eggs cease to segment. If the prevention of segmentation by narcotics were due to the same influence we should have to expect also a lowering of the rate of oxidations to one-third of the normal rate found at 14° C.

3. We tried a number of narcotics, various alcohols, chloral hydrate, chloroform and ethyl urethane. The minimum amount required to suppress cell division permanently was ascertained for each of these narcotics. The minimal dose varies slightly for the eggs of various individuals.

a. Chloral Hydrate.—The minimum amount required to suppress cell division is 4.2 cc. of 0.5 per cent solution (made up in $\frac{M}{2}$ NaCl + KCl + CaCl₂) to 45.8 cc. of sea water. Temperature 15° C.

⁴ Loeb and Wasteneys: *Biochem. Zeitschr.*, xxxvi, p. 355, 1911.

TABLE III.

	OXYGEN CONSUMED	RATE OF OXIDATIONS
	<i>mgm.</i>	
Normal sea water.	0.65	1.00
2.4 cc. 0.5 per cent chloral hydrate in 50 cc. sea water. . . .	0.60	0.92
4.2 cc. 0.5 per cent chloral hydrate in 50 cc. sea water. . . .	0.57	0.88
6.0 cc. 0.5 per cent chloral hydrate in 50 cc. sea water. . . .	0.57	0.88

In the solution with 4.2 cc. and 6.0 cc. of chloral hydrate the eggs were no longer able to segment; but they segmented and developed promptly when transferred to normal sea water. In the solution with 2.4 cc. of chloral hydrate the eggs segmented. The effect of the chloral hydrate upon oxidations was practically nil. The experiment was repeated.

TABLE IV.

	OXYGEN CONSUMED	RATE OF OXIDATIONS
	<i>mgm.</i>	
Normal sea water.	0.51	1.00
4.2 cc. 0.5 per cent chloral hydrate in 50 cc. sea water. . . .	0.42	0.89

Although the segmentations were completely suppressed in this solution the influence of the chloral hydrate was practically negligible. We can say with certainty: The narcotic effect of chloral hydrate upon the egg is not due to asphyxiation or a diminution in the rate of oxidations.

b. Ethyl Urethane.—Three cubic centimeters of a 10 per cent solution (in $\frac{M}{2}$ NaCl + KCl + CaCl₂) in 50 cc. of sea water are sufficient to suppress cell division.

TABLE V.

	OXYGEN CONSUMED	RATE OF OXIDATIONS
	<i>mgm.</i>	
Normal sea water.	0.51	1.00
3 cc. 10 per cent ethyl urethane in 50 cc. sea water.	0.46	0.98

With a few exceptions no eggs segmented in ethyl urethane, but all segmented when put back into normal sea water.

c. *Chloroform*.—Seven cubic centimeters of 0.5 per cent CHCl_3 in $\frac{1}{2}$ $\text{NaCl} + \text{KCl} + \text{CaCl}_2$ were required to suppress cell division.

TABLE VI.

	OXYGEN CONSUMED	RATE OF OXIDATIONS
	<i>mgm.</i>	
Normal sea water	0.47	1.00
43 cc. sea water + 7 cc. 0.5 per cent chloroform	0.41	0.87

No segmentation occurred in the eggs in chloroform, but they segmented and developed normally when transferred to normal sea water. The narcotic effect of chloroform is produced without any considerable lowering of the rate of oxidations. The slight lowering observed is not quite but almost within the limits of error and cannot be considered.

d. *Propyl Alcohol*.—Minimum amount necessary to suppress cell division is 3.5 cc. of 2 M propyl alcohol in 50 cc. of sea water. In order to avoid lowering of concentration of salts the 2 M solution of alcohol was made up in $\frac{1}{2}$ $\text{NaCl} + \text{KCl} + \text{CaCl}_2$.

TABLE VII.

	OXYGEN CONSUMED	RATE OF OXIDATIONS
	<i>mgm.</i>	
In normal sea water	1.00	1.00
2.0 cc. 2 M propyl alcohol in 50 cc. sea water	0.90	0.90
3.5 cc. 2 M propyl alcohol in 50 cc. sea water	0.92	0.92
5.0 cc. 2 M propyl alcohol in 50 cc. sea water	0.93	0.93

In 2 cc. of propyl alcohol a few of the eggs (less than 1 per cent) segmented. In 3.5 cc. and 5 cc. of propyl alcohol no egg segmented. A few underwent cytolysis, but as Warburg has shown, and as we were able to confirm, this does not alter the rate of oxidations in the fertilized egg. We therefore see that the narcotic effect of propyl alcohol is not accompanied by any lowering of the rate of oxidations in the egg.

e. *Various Other Alcohols*.—The narcotic effect of various alcohols follows the rule that each successive alcohol of a series is two or three times as efficient for narcosis as the previous one. The

amount necessary for the suppression of segmentation in the egg of *S. purpuratus* found for the various alcohols was as follows:

	Rate of Efficiency Compared with Methyl Alcohol.
Methyl alcohol, 4.0 cc. 10 M in 50 cc. sea water	1
Ethyl alcohol, 5.0 cc. 4 M in 50 cc. sea water	2
Propyl alcohol, 3.5 cc. 2 M in 50 cc. sea water	6
Butyl alcohol, 3.5 cc. M in 50 cc. sea water	12

TABLE VIII.

	OXYGEN CONSUMED	RATE OF OXIDATIONS
	<i>mgm.</i>	
Normal sea water	0.87	1.00
4 cc. 10 M methyl alcohol in 50 cc. sea water	0.83	0.95
5 cc. 4 M ethyl alcohol in 50 cc. sea water	0.73	0.84
7 cc. $\frac{M}{2}$ butyl alcohol in 50 cc. sea water	0.66	0.76

No eggs segmented in the solutions containing alcohol except in the methyl alcohol in which 25 per cent of the eggs segmented. When transferred to normal sea water all the eggs segmented. The rate of oxidations in these experiments is too high to account for the narcotic effect on the basis of asphyxiation. The experiment with butyl alcohol was repeated.

TABLE IX.

	OXYGEN CONSUMED	RATE OF OXIDATIONS
	<i>mgm.</i>	
Normal sea water	0.84	1.00
6.5 cc. $\frac{M}{2}$ butyl alcohol in 50 cc. sea water	0.66	0.79

4. *Theoretical Remarks.*—The maximal lowering of the rate of oxidations found under the influence of narcotics was 20 per cent; in the majority of cases it was less than this. In the case of propyl alcohol it was less than 10 per cent; in the case of chloral hydrate it was about 10 per cent. Since the temperature coefficient for the rate of oxidations in the eggs is about 2 for 10° C.,⁵ we can produce a lowering of the rate of oxidations of 20 per cent by lowering

⁵ Loeb and Wasteneys: *Biochem. Zeitschr.*, xxxvi, p. 345, 1911.

the temperature two or three degrees, *e. g.*, putting the eggs into sea water of 12° instead of into 15° as in these experiments. The previous experiments of Loeb have shown that the eggs of *S. purpuratus* segment not only at 12° but even at 3° C., when the rate of oxidations is less than one-half of that observed at 15° C. In addition, the experiments with KCN also show that this substance does not suppress cell division until the rate of oxidations is reduced to one-third of the normal value. From these facts we can state with certainty that the effect of narcotics upon the eggs of the sea urchin is not due to asphyxiation; a conclusion which Warburg reached also in his experiments with phenyl urethane.

Kisch⁶ has recently published experiments which show that the photodynamic effect due to oxidations is raised instead of being diminished if narcotics are added to the medium.

SUMMARY.

It is shown that chloral hydrate, ethyl urethane, chloroform and various alcohols produce complete narcosis in the fertilized eggs of the sea urchin without practically lowering the rate of oxidations in the egg.

⁶ Kisch: *Zeitschr. f. Biol.*, ix, p. 399, 1913.

ÜBER DIE ANPASSUNG VON FUNDULUS AN HÖHERE KONZENTRATIONEN.*

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I.

i. Unter Anpassung versteht man bekanntlich die Tatsache, dass die Änderung der Lebensbedingungen eines Organismus dessen Struktur oder Konstitution in dem Sinne ändert, dass er die neuen Lebensbedingungen besser erträgt.

Versuche über die Anpassung an höhere Temperaturen und an höhere Konzentrationen der umgebenden Lösungen haben ergeben, dass diese Anpassung nur oder besser dann stattfindet, wenn die Änderung der Konzentration oder der Temperatur allmählich erfolgt, während eine rasche Änderung zum Tode führt.

Bei Versuchen, die Loeb und Wasteneys¹⁾ über die Anpassung von Fundulus an höhere Temperaturen jüngst veröffentlichten, machten sie die Beobachtung, dass dieser Fisch eine höhere Temperatur ertragen kann, wenn er in $\frac{m}{8}$ -Lösung von NaCl + KCl + CaCl₂ ist, als wenn er in einer niedrigeren Konzentration, etwa $\frac{m}{64}$, ist; obwohl die Fische bei gewöhnlicher Temperatur beliebig lange in einer solchen Lösung leben. Das legte den Gedanken nahe, dass vielleicht eine Änderung der Permeabilität der Kiemen des Fisches bei diesen Anpassungserscheinungen im Spiele sei. Das regte uns dazu an, Versuche über die Anpassung derselben Fische an höhere Konzentrationen anzustellen. Diese Versuche waren um so mehr von Interesse, da Fundulus in weiten Grenzen von der Konzentration des umgebenden Mediums unabhängig ist und es deshalb

* Eingegangen am 28. Mai 1913.

¹⁾ Loeb und Wasteneys, Journ. experim. Zool. 21, 543, 1912.

von Interesse war, zu sehen, wie weit die Anpassung an höhere Konzentrationen bei diesem Tier getrieben werden kann.

Eine Zusammenstellung der Literatur über die Anpassung von Organismen an höhere Konzentrationen findet sich in einer Arbeit von Dernoscheck¹). Dernoscheck hat unter der Leitung von W. o. Ostwald Versuche an Daphnien angestellt und ist zu dem Schluss gekommen, dass für die Tatsachen der Anpassung an höhere Konzentrationen eine rein osmotische Erklärung unzureichend ist, und dass die Erfahrungen über antagonistische Salzwirkungen mit zu berücksichtigen sind. Wir werden sehen, dass unsere Erfahrungen diesen Schluss unterstützen.

2. Die Frage, die wir beantworten wollten, war in erster Linie, wie es kommt, dass die rasche Änderung einer Lebensbedingung (z. B. Temperatur, Konzentration der umgebenden Lösung) den Tod eines Organismus herbeiführt, während eine langsamere Änderung derselben Lebensbedingung um denselben Betrag ohne Schaden ertragen wird.

Wir untersuchten den Mechanismus der Anpassung dieser Fische an höhere Konzentrationen. Bei der Einfachheit der Versuche dürfen wir die Resultate wohl in aller Kürze mitteilen. Jeder der hier mitgeteilten Versuche ist sehr oft wiederholt worden.

Eine Mischung von $\text{NaCl} + \text{KCl} + \text{CaCl}_2$, in dem Verhältnis in dem diese Salze im Seewasser enthalten sind, bezeichnen wir als künstliches Seewasser. Die zu den Versuchen benutzten Fische, die natürlicherweise im Seewasser leben, waren weniger als 1 Jahr alt. Je 4 Fische wurden in eine Reihe zylindrischer Gefässe mit je 500 ccm Lösung gebracht. Für jeden Anpassungsversuch wurden 100 oder mehr Fische benutzt.

II.

3. Bringen wir Fische aus natürlichem Seewasser in künstliches Seewasser höherer Konzentration, so finden wir, dass dieselben in wenigen Stunden sterben, wenn die Konzentration $^{10}/_8$ m oder höher ist; dass sie aber in $^9/_8$ m und darunter leben können. Man kann aber alle Fische in $^{10}/_8$ m dauernd am Leben erhalten, wenn

¹) Dernoscheck, Arch. f. d. ges. Physiol. 143, 303, 1911.

man folgendermassen verfährt. Die Fische werden in 500 ccm $\frac{m}{2}$ künstliches Seewasser gebracht, dem man nach folgenden Intervallen folgende Mengen von $2\frac{1}{2}$ m künstliches Seewasser zufügt:

	Nach	24	Stunden	50 ccm	$2\frac{1}{2}$ m	künstliches	Seewasser
nach	weiteren	24	"	50	" $2\frac{1}{2}$	"	"
"	"	24	"	25	" $2\frac{1}{2}$	"	"
"	"	24	"	25	" $2\frac{1}{2}$	"	"

Lässt man die Verdunstung unberücksichtigt, so ist jetzt die Lösung ungefähr m. Von hier können die Fische ohne Schaden in eine $\frac{10}{8}$ m-Lösung von $\text{NaCl} + \text{KCl} + \text{CaCl}_2$ gebracht werden. Sie bleiben in einer solchen Lösung dauernd (beobachtet über 1 Monat) am Leben; sie sind also nunmehr an eine Konzentration „angepasst“, in der sie bei plötzlicher Übertragung in wenigen Stunden sterben.

Bei dem angegebenen Verfahren bleiben praktisch alle Fische am Leben. Steigert man die Konzentration rascher, so sterben eine Reihe von Fischen im Laufe der Anpassung, und um so mehr je rascher die Konzentrationssteigerung.

4. Bringt man Fische in der vorhin angegebenen Weise allmählich in eine m-Lösung und lässt man sie in dieser Lösung so nimmt die Konzentration der Lösung durch Verdunstung sehr langsam zu. Man kann nun prüfen, bis zu welcher Konzentration die Fische am Leben bleiben. Die Fische starben rasch ab, als das spezifische Gewicht der Lösung 1,0644 überstieg. Das würde also etwas höher als $\frac{12}{8}$ m- $\text{NaCl} + \text{KCl} + \text{CaCl}_2$ sein. Wir dürfen wohl sagen, dass die Fische an eine Lösung von künstlichem Seewasser, die höher ist als $\frac{12}{8}$ m, nicht mehr durch die von uns gewählte Methode angepasst werden können. Vermutlich ist die maximale Grenze noch etwas niedriger.

5. Wenn man Fische in der in 3. beschriebenen Weise vorbehandelt und sie in der m-Lösung stehen lässt, so nimmt die Konzentration der letzteren durch Verdunstung langsam zu. Es lässt sich zeigen, dass zunächst mit Zunahme der Konzentration auch die Widerstandsfähigkeit der Fische gegen höhere Konzentrationen zunimmt.

Eine Partie Fische war vom 24. III. bis 28. III., eine zweite vom 3. IV. bis 7. IV. auf $m\text{-NaCl} + \text{KCl} + \text{CaCl}_2$ gebracht worden. Am 8. IV. war das spezifische Gewicht der Lösung der ersten Gruppe 1,0586 (also Konzentration etwas höher als $^{10}/_8 m$), und das der zweiten Gruppe 1,0439 (also Konzentration etwas höher als $^8/_8 m$). Je 4 Fische jeder Gruppe wurden dann in 500 ccm $^{10}/_8, ^{11}/_8, ^{12}/_8, ^{13}/_8, ^{14}/_8, ^{15}/_8 m$ künstliches Seewasser gebracht und ihre Lebensdauer bestimmt. Es zeigte sich, dass die Fische, die in $^{10}/_8 m$ gewesen waren, erheblich resistenter waren als die Fische, die in $^8/_8 m$ gewesen waren. Tabelle I gibt die Zahl der überlebenden Fische nach 24 Stunden.

TABELLE I.

Angepasst an	Zahl der nach 24 Stunden überlebenden Fische in					
	$^{10}/_8$	$^{11}/_8$	$^{12}/_8$	$^{13}/_8$	$^{14}/_8$	$^{15}/_8 m$
	Seewasser					
$^{10}/_8 m$	4	4	4	1	1	0
$^8/_8 m$	4	4	1	0	0	0

Die auf $^{10}/_8 m$ angepassten Fische blieben in $^{11}/_8 m$ längere Zeit am Leben; die auf $^8/_8 m$ angepassten blieben nur in $^{10}/_8 m$ am Leben.

Zur Kontrolle waren gleichzeitig Fische direkt aus Seewasser in dieselben Lösungen gebracht worden. In 3 Stunden waren alle tot; auch die in $^{10}/_8 m$.

Ein zweites umfassenderes Beispiel möge noch angeführt werden. Eine grössere Zahl von Fischen wurde am 5. III. in verschiedene Lösungen gebracht, deren Konzentrationen verschieden rasch erhöht wurden, so dass am 18. III. je eine Zahl von Fischen in folgenden Lösungen vorhanden war: $^m/_4, ^6/_8 m, ^8/_8 m, ^{10}/_8 m, ^{11}/_8 m$ und $^{12}/_8 m\text{-NaCl} + \text{KCl} + \text{CaCl}_2$. Diese Fische wurden in folgende Lösungen gebracht: $^{10}/_8, ^{11}/_8, ^{12}/_8, ^{13}/_8$ und $^{14}/_8 m$. Tabelle II gibt die Zahl der überlebenden Fische nach 48 Stunden. Je vier Fische in einem Gefäss.

3 Tage später waren alle Fische in $^{13}/_8$ und $^{14}/_8 m$ tot und die in $^{12}/_8 m$ starben den folgenden Tag. Am längsten lebten die Fische, die in $^8/_8 m$ gewesen und in $^{10}/_8 m$ gebracht waren.

TABELLE II.

Angepasst an	Zahl der überlebenden Fische nach 48 Stunden in				
	$10/8$	$11/8$	$12/8$	$13/8$	$14/8$ m-
	NaCl + KCl + CaCl ₂				
$m/4$	0	0	0	0	0
$6/8$ m	4	0	0	0	0
$8/8$ m	4	4	1	0	0
$10/8$ m	4	3	3	0	0
$11/8$ m	—	4	2	1	0
$12/8$ m	—	—	4	2	3

Wir dürfen daraus schliessen, dass die günstige Veränderung, die das Wesen der Anpassung ausmacht, mit zunehmender Erhöhung der Konzentration zunimmt; bis die Grenzkonzentration erreicht wird.

6. *Fundulus* kann ohne Schaden aus Seewasser in sehr niedrige Konzentrationen und auch in Süsswasser übertragen werden. Es war von Interesse zu ermitteln, ob Fische, die mehrere Wochen in $10/8$ m-NaCl + KCl + CaCl₂ gelebt haben, diese Immunität gegen sehr verdünnte Lösungen noch besitzen. Das ist der Fall. Fische, die mehrere Wochen in $10/8$ m-Seewasser gelebt haben, können unmittelbar und ohne Schaden in $m/80$ -NaCl + KCl + CaCl₂ übertragen werden. Sie leben beliebig lange in dieser Lösung. Auch wenn sie in gewöhnliches Wasserleitungswasser übertragen werden, so leben sie darin ebenso lange und ebenso gut, wie die aus Seewasser übertragenen. Durch Anpassung an höhere Konzentrationen wird also die Toleranz gegen niedere Konzentrationen von *Fundulus* nicht beeinträchtigt.

7. Diese Beobachtungen sowie unsere früheren Erfahrungen über die Anpassung dieser Fische an höhere Temperaturen legten den Gedanken nahe, zu untersuchen, ob die Anpassung an höhere Konzentrationen irreversibel sei. Zu dem Zweck wurden Fische erst an $10/8$ m-Seewasser angepasst und dann in normales oder verdünntes Seewasser zurückgebracht, um zu sehen, ob sie die erworbene Anpassung wieder verlieren würden. Diese Versuche ergaben, dass die Anpassung längere Zeit erhalten bleibt. Die Konzentration des Seewassers wurde wie gewöhnlich langsam auf m erhöht, und 4 Tage später wurde die Hälfte der angepassten Fische in $m/8$ -Seewasser gebracht. Am folgenden Tage wurden je 4 Fische der beiden

Partien in $12/8$, $13/8$ und $14/8$ m-Seewasser gebracht. Die Fische, die einen Tag in der $m/8$ -Lösung gewesen waren, lebten ebenso lange in den $12/8$, $13/8$ und $14/8$ m-Lösungen, wie die Fische, die direkt aus der m-Lösung genommen wurden; d. h. es dauerte über 3 Tage, bis alle Fische in den $12/8$ bis $14/8$ m-Lösungen tot waren. Bringt man nicht angepasste Fische aus normalem Seewasser in diese Lösungen, so sterben sie alle in wenigen (2 bis 4) Stunden.

Dieser Versuch beweist auch des weitern, dass, wenn die Tiere einmal an eine hohe Konzentration angepasst sind, sie ohne Schaden den plötzlichen Wechsel aus niederer in hohe Konzentration und umgekehrt ertragen.

Der rasche Wechsel von niederer auf hohe ($10/8$ m-) Konzentration ist nur für nicht angepasste Organismen schädlich.

8. Bei der weiteren Fortsetzung dieser Versuche ergab sich nun, dass, wenn für höhere Konzentration angepasste Tiere in Lösungen niederer Konzentration zurückgebracht werden, doch allmählich eine Abschwächung der erworbenen Anpassung für höhere Konzentration eintritt, die sich versuchsmässig nachweisen lässt. Es scheint ferner, dass diese Abschwächung um so rascher erfolgt, je niedriger die Konzentration ist, in welche die Fische nach der erworbenen Anpassung zurückgebracht werden. Der folgende Versuch möge als Beispiel dienen.

TABELLE III.

Nach	Zahl der überlebenden Fische in $10/8$ m-Seewasser			
	Fische für $10/8$ m adaptiert, aber 2 Tage in $m/8$ - $\text{NaCl} + \text{KCl} + \text{CaCl}_2$			Nicht adaptierte Fische
	$m/80$ -	$m/8$ -	$m/2$ -	
6 Stunden	2	2	2	0
2 Tagen	2	2	2	0
4 "	0	2	1	0
6 "	0	1	0	0
7 "	0	0	0	0

Eine grössere Partie von Fischen wurde in der gewöhnlichen Weise vom 1. IV. bis 4. IV. an m-NaCl + KCl + CaCl_2 angepasst. Am 12. IV. wurden diese Fische in folgende drei Lösungen verteilt: $m/80$ -, $m/8$ - und $m/2$ -NaCl + KCl + CaCl_2 . Nach 2, 4 und 7 Tagen

wurden je 2 Fische aus jeder dieser Lösungen in $10/8$ m-NaCl + KCl + CaCl₂ gebracht, um zu sehen, ob sie noch in dieser Lösung zu leben vermögen. Zur Kontrolle wurden jedesmal auch 2 nicht angepasste Fische aus normalem Seewasser in diese konzentrierten Lösungen gebracht.

TABELLE IV.

Nach	Zahl der überlebenden Fische in $10/8$ m-Seewasser			
	Fische für $10/8$ m adaptiert, aber 4 Tage in $m/80$ - $m/8$ - $m/2$ - NaCl + KCl + CaCl ₂			Nicht adaptierte Fische
6 Stunden	2	2	2	0
2 Tagen	0	2	2	0
4 "	0	0	1	0
5 "	0	0	0	0

Das Resultat ist völlig eindeutig: Auch nach 7 tägigem Aufenthalt der Fische in der verdünnten Lösung ist noch eine deutliche Resistenz gegen die $10/8$ m-Lösung vorhanden; denn die nicht vorbehandelten Tiere sterben in dieser Lösung in weniger als 6 Stunden, während die vorbehandelten Tiere alle länger leben. Es ist ferner

TABELLE V.

Nach	Zahl der überlebenden Fische in $10/8$ m-Seewasser			
	Fische für $10/8$ m adaptiert, aber 7 Tage in $m/80$ - $m/8$ - $m/2$ - NaCl + KCl + CaCl ₂			Nicht adaptierte Fische
6 Stunden	2	2	2	0
2 Tagen	0	2	1	0
3 "	0	0	0	0

deutlich, dass die Adaptierung in $m/80$ -NaCl + KCl + CaCl₂ rascher abgeschwächt wird als in $m/8$ - und $m/2$ -Lösungen derselben Salze. Dass aber auch bei den Fischen, die in die $m/8$ - und $m/2$ -Lösungen gebracht waren, eine deutliche Abnahme der Resistenz gegen hohe Konzentrationen eintrat, ist dadurch bewiesen, dass die einmal an $10/8$ m angepassten Fische in dieser Lösung beliebig lange am Leben bleiben, wenn man sie dauernd in dieser Lösung lässt.

9. Es wurden Versuche angestellt, um zu ermitteln, ob schon eine

Abnahme der Widerstandsfähigkeit zu bemerken ist, wenn man die an $10/8$ m adaptierten Fische nur auf 3 Stunden in eine verdünnte Lösung bringt. Es stellte sich heraus, dass, wenn adaptierte Fische auf 3 Stunden in eine $m/8$ -Lösung von $\text{NaCl} + \text{KCl} + \text{CaCl}_2$ gebracht werden, keine Spur einer Abnahme der Anpassung wahrnehmbar ist.

III.

10. Die theoretischen Erwägungen, zu denen die bisher erwähnten Versuche führten, legten den Gedanken nahe, zu untersuchen, ob Fische, die für höhere Konzentrationen ($10/8$ m) von $\text{NaCl} + \text{KCl} + \text{CaCl}_2$ angepasst sind, damit auch für höhere Konzentrationen solcher Salzlösungen angepasst sind, die in viel niedrigerer Konzentration schädlich wirken als $\text{NaCl} + \text{KCl} + \text{CaCl}_2$. Zunächst wurden reine NaCl -Lösungen untersucht. Fische, die an künstliches Seewasser so weit angepasst waren, dass sie beliebig lange in $10/8$ m- $\text{NaCl} + \text{KCl} + \text{CaCl}_2$ lebten, wurden in $6/8$, $7/8$ und $8/8$ m- NaCl gebracht, um festzustellen, ob sie in diesen Lösungen länger leben als Fische, die direkt aus normalem Seewasser genommen werden. Ehe die Fische in die NaCl -Lösungen gebracht wurden, wurden sie zweimal in $m/8$ - NaCl -Lösung gewaschen; d. h. sie wurden frei von Seewasser (mittels eines Porzellantrichters) in eine $m/8$ - NaCl -Lösung gebracht, in der sie 15 Minuten blieben, und dann in eine zweite $m/8$ - NaCl -Lösung ebenfalls auf 15 Minuten gebracht. Das war nötig, um das äusserlich am Fisch haftende CaCl_2 loszuwerden, da ja geringe Mengen von CaCl_2 bereits entgiftend auf NaCl wirken.

Die Versuche ergaben das Resultat, dass die für höhere Konzentrationen von $\text{NaCl} + \text{KCl} + \text{CaCl}_2$ angepassten Tiere auch in höheren Konzentrationen von reinen NaCl -Lösungen etwas länger leben als die nicht angepassten Tiere.

Die ursprüngliche Zahl der Fische, die in jede der Lösungen gebracht wurden, betrug 4. Die Fische waren auf $8/8$ m-Seewasser gebracht worden und blieben in dieser Lösung 4 Tage. Dann wurden sie in $6/8$, $7/8$ und $8/8$ m- NaCl gebracht. Zur Kontrolle wurden gleichzeitig nicht vorbehandelte Fische in reine NaCl -Lösungen derselben Konzentrationen gebracht. Die letzteren Fische waren

in 2 Stunden alle tot. Das Verhalten der anderen wird in Tabelle VI erwähnt.

TABELLE VI.

Nach	Zahl der überlebenden, auf $10/8$ m-Seewasser adaptierten Fische in		
	$6/8$	$7/8$	$8/8$ m-NaCl
4 Stunden	4	4	3
1 Tage	4	2	0
2 Tagen	3	1	0
3 "	3	0	0

Während also die nicht angepassten Fische in $6/8$ m-NaCl in 2 Stunden starben, überlebten die auf $10/8$ m angepassten Fische in dieser Lösung mehr als 3 Tage.

In einem zweiten Falle waren Fische in 4 Tagen auf $8/8$ m-NaCl + KCl + CaCl₂ gebracht worden und 2 Tage später der NaCl-Lösung ausgesetzt worden. Tabelle VII gibt die Zahl der angepassten Tiere, die in den verschiedenen Lösungen überlebten.

TABELLE VII.

Nach	Zahl der überlebenden, auf $8/8$ m-Seewasser adaptierten Fische in		
	$6/8$	$7/8$	$7/8$ m-NaCl
4 Stunden	4	4	3
1 Tage	3	2	1
2 Tagen	2	1	0

Die zur Kontrolle in dieselben Lösungen gebrachten nicht vorbehandelten Fische starben alle in weniger als 4 Stunden. Diese Versuche wurden oft mit demselben Erfolg wiederholt. Wir dürfen also sagen, dass die für Seewasser höherer Konzentrationen angepassten Fische länger in reinen NaCl-Lösungen höherer Konzentrationen leben als die nicht angepassten Fische.

II. Die früheren Versuche des Verfassers hatten gezeigt, dass in reinen NaCl-Lösungen von $m/8$ oder noch geringerer Konzentration *Fundulus* beliebig lange leben kann, dass aber eine $m/4$ -NaCl-Lösung schon als giftig angesehen werden muss, da die Fische hier nach einiger Zeit sterben. Es fragte sich nun, ob die an $10/8$ m-Seewasser angepassten Fische auch in $m/4$ - oder $m/2$ -NaCl-Lösungen länger

leben als die nicht angepassten. Diese Versuche des Verfassers haben zu keinem entscheidenden Resultat geführt.

12. Ich versuchte nun, ob auf noch anderem Wege eine Anpassung der Fische an höhere Konzentrationen von NaCl möglich sei. Die Versuche über die Entgiftung von NaCl durch CaCl_2 bei *Fundulus* legten den Gedanken nahe, dass die grössere Widerstandsfähigkeit angepasster Fische gegen reine NaCl-Lösungen vielleicht dadurch bedingt sei, dass dieselben einen stärkeren Niederschlag eines unlöslichen Calciumsalzes an ihrer Oberfläche besitzen. Um diese Ansicht zu prüfen, wurde der Versuch gemacht, Fische gegen reine NaCl-Lösungen durch Vorbehandlung mit CaCl_2 widerstandsfähiger zu machen. Es wurden deshalb Fische aus Seewasser in Schalen mit 500 ccm $\frac{m}{8}$ -NaCl + KCl + CaCl_2 gebracht, dem noch 10 ccm $2\frac{1}{2}$ m- CaCl_2 zugesetzt wurden. Die absolute molekulare Konzentration dieser Lösung war also nur ungefähr $\frac{1}{3}$, der Calciumgehalt war aber ungefähr 6 mal so gross wie der des Seewassers.

Die Fische waren vor dem Versuch 2 Tage in dieser Lösung gewesen. Ihre Widerstandsfähigkeit gegen $\frac{6}{8}$, $\frac{7}{8}$ und $\frac{8}{8}$ m-NaCl wurde mit derjenigen verglichen, welche Fische besaßen, die an $\frac{10}{8}$ m-Seewasser adaptiert waren. Gleichzeitig wurde auch ein Kontrollversuch mit nicht adaptierten Fischen angestellt. Die letzteren waren nach 6 Stunden alle tot. In jede Lösung wurden 4 Fische gebracht.

TABELLE VIII.

Nach	Zahl der überlebenden Fische in			
	$\frac{6}{8}$	$\frac{7}{8}$	$\frac{8}{8}$ m-NaCl	
6 Stunden	3	0	0	Vorbehandelt mit Ca ¹⁾
	4	2	0	" " $\frac{10}{8}$ m-Seewasser
1 Tage	1	0	0	" " Ca
	4	1	0	" " $\frac{10}{8}$ m-Seewasser
4 Tagen	1	0	0	" " Ca
	2	0	0	" " $\frac{10}{8}$ m-Seewasser
5 "	0	0	0	" " Ca
	1	0	0	" " $\frac{10}{8}$ m-Seewasser

¹⁾ Alle Fische wurden erst durch zweimaliges Waschen in $\frac{m}{8}$ -NaCl von allen Spuren von Ca befreit, welche die Oberfläche oder die Kiemen benetzten.

Aus diesem Versuch geht hervor, dass Vorbehandlung mit Ca etwas Schutz gegen höhere Konzentration von NaCl verleiht, dass aber der Schutz durch die Vorbehandlung mit $10/8$ m-Seewasser merklich grösser ist.

13. Es wurde dann der Versuch gemacht, ob es möglich sei, Fische dadurch gegen höhere Konzentrationen von reinen NaCl-Lösungen zu immunisieren, dass man sie mit reinen NaCl-Lösungen behandelte. Man spricht ja in gewissen Fällen von einer „Gewöhnung an Gifte“, und es wurde der Versuch gemacht, ob diese „Gewöhnung“ auch für Salze bestehe. Es wurden deshalb Fische längere Zeit in eine $3/8$ und $3/4$ reine NaCl-Lösung gebracht und dann ihre Widerstandsfähigkeit gegen $6/8$, $7/8$ und $8/8$ m mit derjenigen der nicht vorbehandelten Fische verglichen. Es trat in keinem Falle durch die Vorbehandlung mit reinen NaCl-Lösungen eine Erhöhung der Widerstandskraft gegen NaCl-Lösungen höherer Konzentrationen ein, sondern eher das Gegenteil¹⁾.

14. Dieser Versuch wurde in folgender Weise abgeändert. Fische wurden, wie am Eingang dieses Aufsatzes geschildert wurde, allmählich vom 12. II. bis 16. II. in eine m-Lösung von NaCl + KCl + CaCl₂ gebracht. Aus dieser Lösung wurde ein Teil derselben an den darauf folgenden Tagen in Lösungen mit zunehmend höherem NaCl-Gehalt, aber demselben osmotischen Druck gebracht, nämlich:

am 17. II.: in 100 ccm $2\frac{1}{2}$ m-NaCl + 100 ccm $2\frac{1}{2}$ m-NaCl + KCl + CaCl₂ + 300 ccm H₂O,

am 18. II.: in 150 ccm $2\frac{1}{2}$ m-NaCl + 50 ccm $2\frac{1}{2}$ m-NaCl + KCl + CaCl₂ + 300 ccm H₂O,

am 19. II.: in 175 ccm $2\frac{1}{2}$ m-NaCl + 25 ccm $2\frac{1}{2}$ m-NaCl + KCl + CaCl₂ + 300 ccm H₂O.

Es wurde nun untersucht, ob diese Tiere eine höhere Widerstandskraft gegen $6/8$, $7/8$ und $8/8$ m-NaCl-Lösung besaßen, als Fische desselben Versuchs, die dauernd in der $8/8$ m-Lösung geblieben waren. Das war nicht der Fall, wie folgende Tabelle zeigt. Je 4 Fische wurden in eine Lösung gebracht.

¹⁾ Nebenbei sei auch bemerkt, dass es nicht gelang, durch Vorbehandlung der Fische mit CaCl₂-Lösungen niedriger Konzentration dieselben an höhere Konzentrationen von CaCl₂ anzupassen.

TABELLE IX.

Nach	Zahl der überlebenden Fische in			
	$\frac{6}{8}$	$\frac{7}{8}$	$\frac{8}{8}$ m-NaCl	
5 Stunden	4	1	3	Aus $\frac{8}{8}$ m-Seewasser
	2	0	0	" 175 ccm NaCl + 25 ccm NaCl + KCl + CaCl ₂
1 Tage	4	0	1	" $\frac{8}{8}$ m-Seewasser
	0	0	0	" 175 ccm NaCl + 25 ccm NaCl + KCl + CaCl ₂
2 Tagen	2	0	0	" $\frac{8}{8}$ m-Seewasser
	0	0	0	" 175 ccm NaCl + 25 ccm NaCl + KCl + CaCl ₂

Die zur Kontrolle ohne Vorbehandlung direkt aus normalem Seewasser in $\frac{6}{8}$, $\frac{7}{8}$ und $\frac{8}{8}$ m-NaCl gebrachten Fische starben in 5 Stunden.

Dieser und ähnliche Versuche beweisen, dass Erhöhung des NaCl-Gehaltes in der $\frac{8}{8}$ m-Lösung die Widerstandskraft der Fische gegen reine NaCl-Lösungen herabsetzt.

Aus diesen und ähnlichen Versuchen müssen wir schliessen, dass es nicht gelingt, durch Vorbehandlung mit NaCl die Widerstandskraft der Fische gegen NaCl-Lösungen zu erhöhen. Eine „Gewöhnung“ der Fische an reines NaCl fand also nicht statt.

15. Es wurden Versuche angestellt, ob Fische, die bis auf m-Seewasser gebracht waren, auch gegen höhere Konzentrationen von Na₂SO₄ widerstandsfähiger seien als nicht angepasste Fische. Die Konzentration reiner Na₂SO₄-Lösung, die Fische ertragen können, schwankt etwas, im allgemeinen aber sterben sie rasch in Lösungen, die $\frac{m}{8}$ Na₂SO₄ übersteigen. Fundulus, die seit 2 Wochen auf $\frac{10}{8}$ m-Seewasser angepasst waren, wurden in reine Lösungen von Na₂SO₄ verschiedener Konzentration übertragen, und gleichzeitig nicht angepasste Fische in dieselben Lösungen. Die Fische wurden erst zweimal in $\frac{m}{20}$ -Na₂SO₄ gewaschen, ehe sie in die Lösungen gebracht wurden.

TABELLE X.

Nach	Zahl der überlebenden Fische in					
	$\frac{3}{20}$	$\frac{7}{40}$	$\frac{4}{20}$	$\frac{9}{40}$	$\frac{8}{20}$ m-Na ₂ SO ₄	
1 Tage	4	4	2	2	0	Angepasst
	0	0	0	0	0	Nicht angepasst
2 Tagen	4	4	2	0	0	Angepasst
	0	0	0	0	0	Nicht angepasst

Am nächsten Tage starben aber auch die angepassten Fische. Eine Wiederholung des Versuchs bestätigte das Resultat.

Es zeigt sich also, dass Fische, die für Seewasser von höherer Konzentration angepasst sind, auch damit eine erhöhte Widerstandskraft gegen höhere Konzentrationen von Na_2SO_4 besitzen. Es sei ausdrücklich darauf hingewiesen, dass die absoluten Konzentrationen von Na_2SO_4 , die in diesem Versuche in Anwendung kamen, geringer sind als die des Seewassers. Es kann sich also nicht in diesem Versuche um eine Erhöhung der Widerstandsfähigkeit gegen etwaigen Wasserverlust von seiten des Fisches handeln.

16. Schliesslich wurden auch noch Versuche über den Widerstand von adaptierten und nicht adaptierten *Fundulus* gegen höhere Konzentrationen von NaNO_3 angestellt. NaNO_3 ist erheblich giftiger als Na_2SO_4 , und um mit höheren Konzentrationen von NaNO_3 arbeiten zu können, wurde es zum Teil mit $\text{NaCl} + \text{KCl} + \text{CaCl}_2$ entgiftet, d. h. NaNO_3 wurde in $\frac{m}{8}$ -Lösungen dieser letzteren drei Salze anstatt in destilliertem Wasser gelöst. Tabelle XI gibt ein Beispiel. Fische, die seit einer Woche an $\frac{10}{8}$ m-Seewasser angepasst waren, wurden mit nicht angepassten Fischen verglichen. Je 4 Fische wurden in eine Lösung gebracht.

TABELLE XI.

Nach	Zahl der überlebenden Fische in							
	15 ccm	20 $2\frac{1}{2}$ m- NaNO_3	25 m- NaNO_3	30 in 500 ccm	35 $\frac{m}{8}$ -Seewasser	40	45	
2 Tagen	4	4	4	3	3	3	0	Angepasst
	4	3	4	1	1	0	0	Nicht angepasst
4 "	4	4	2	3	1	1	0	Angepasst
	4	2	1	0	0	0	0	Nicht angepasst
6 "	4	4	2	2	0	0	0	Angepasst
	3	2	0	0	0	0	0	Nicht angepasst

Wie man sieht, sind die angepassten Fische widerstandsfähiger gegen die höheren Konzentrationen von NaNO_3 als die nicht angepassten. In einer früheren Arbeit haben wir gezeigt, dass die Fische in NaNO_3 bald Vergiftungserscheinungen zeigen und auf der Seite oder dem Rücken liegen. Diese Symptome treten bei den angepassten Tieren ebenso früh ein wie bei den nicht angepassten Tieren.

Dieser Versuch wurde mehrfach mit demselben Resultat wiederholt.

THEORETISCHE BEMERKUNGEN.

Wenn wir *Fundulus* plötzlich aus normalem Seewasser in $10/8$ m künstliches Seewasser bringen, so sterben die Fische in wenigen Stunden. Erhöhen wir aber die Konzentration des Seewassers nur langsam, so leben sie beliebig lange und ohne Schaden zu leiden in Seewasser. Da ich im vorigen Sommer beobachtet habe, dass Eier von *Fundulus* in $10/8$ m, ja selbst in $11/8$ m-Seewasser noch auskriechen können, so dürfen wir sagen, dass solche Fische dauernd in $10/8$ m-Seewasser werden leben können.

Wie sollen wir es uns vorstellen, dass eine langsame Erhöhung der Konzentration die Tiere nicht schädigt, während eine plötzliche Erhöhung die Tiere tötet? Das wird klar, wenn wir annehmen, dass die Erhöhung der Konzentration des Seewassers zwei verschiedene Wirkungen ausübt, eine schädigende und eine modifizierende oder reparierende; und wenn wir zweitens annehmen, dass die reparierende oder modifizierende relativ langsam erfolgt, während die schädigende Wirkung um so rascher erfolgt und um so stärker ist, je grösser der Konzentrationsunterschied. Erfolgt nun die Erhöhung der Konzentration langsam, so kann die modifizierende oder reparierende Wirkung mit der schädigenden Schritt halten, oder richtiger, die Ausbesserung des kleinen Schadens, den die langsame Konzentrationserhöhung bewirkt, kann stattfinden, ehe der Schaden dem Tiere gefährlich wird. Findet aber plötzlich eine sehr hohe Konzentrationserhöhung statt, so ist die Schädigung so stark, dass das Tier stirbt, ehe die reparierende Wirkung Zeit hat, den ganzen Schaden auszubessern.

Über die Natur der schädigenden Wirkung der höheren Konzentration können wir nichts aussagen. Beobachtungen an Seeigeln zeigen, dass jede Salzlösung zerstörend (cytolytisch) wirkt, wenn sie nur eine genügende Konzentration erreicht. Diese Konzentration ist für physiologisch-äquilibrierte Lösungen höher als für nicht äquilibrierte. Noch weniger wissen wir über die Natur der reparierenden Wirkung. Am einfachsten wäre es, anzunehmen, dass die Salze mit der Oberflächenlamelle des Fisches (der Kiemen)

chemisch reagieren und dabei die Widerstandskraft dieser Lamelle ändern; $\text{NaCl} + \text{KCl} + \text{CaCl}_2$ üben eine Art „gerbende Wirkung“ aus, die nur langsam reversibel ist, und diese Gerbung ist um so gründlicher, je höher innerhalb gewisser Grenzen die Konzentration der Lösung. Lösungen von höherer Konzentration als $12/8$ m schädigen unter allen Umständen. Ist die Oberflächenlamelle einmal mit $10/8$ m-Seewasser gegerbt, so erträgt der Fisch eine plötzliche Übertragung aus H_2O in $10/8$ m-Seewasser und umgekehrt ohne Schaden.

In diesem Zustande ist der Fisch auch etwas widerstandsfähiger gegen reine Lösungen von NaCl , NaNO_3 und Na_2SO_4 und vermutlich viele andere schädliche Salze.

Von grosser Bedeutung für alle Theorien der Anpassung ist die Tatsache, dass auch im letzteren Falle die Widerstandsfähigkeit gegen höhere Konzentrationen nur wenig erhöht werden kann, dass also die Anpassungsfähigkeit sehr beschränkt ist.

ZUSAMMENFASSUNG DER RESULTATE.

1. Es wird gezeigt, dass, wenn man *Fundulus* aus Seewasser plötzlich in $10/8$ m-Seewasser bringt, der Fisch in wenigen Stunden stirbt. Bringt man ihn aber im Laufe von 2 oder 3 Tagen allmählich in $10/8$ m-Seewasser, so kann der Fisch dauernd in der Lösung leben.

2. Diese Anpassung beruht auf einer nur langsam reversiblen Änderung. Denn wenn der angepasste Fisch in Seewasser oder verdünntes Seewasser zurückgebracht wird, so behält er seine Anpassung eine Reihe von Tagen. Er kann innerhalb dieser Zeit ohne jeden Schaden jederzeit plötzlich aus sehr verdünnten Lösungen in $10/8$ m-Seewasser gebracht werden und umgekehrt.

3. Ist ein Fisch einmal für $10/8$ m-Seewasser angepasst, so ist gleichzeitig auch seine Widerstandsfähigkeit gegen Lösungen von Na_2SO_4 , NaNO_3 und NaCl erhöht. Da die beiden ersteren Lösungen in niedrigerer Konzentration angewendet werden wie das Seewasser, so kann es sich nicht um eine Anpassung an „Wasserverlust“ handeln.

4. Während es möglich ist, durch Erhöhung der Konzentration

von $\text{NaCl} + \text{KCl} + \text{CaCl}_2$ den Widerstand der Fische gegen eine reine NaCl -Lösung zu erhöhen, gelingt das gleiche nicht durch eine Vorbehandlung mit einer reinen NaCl -Lösung. Wohl aber gelingt eine wenn auch geringere Widerstandserhöhung gegen eine reine NaCl -Lösung durch eine Vorbehandlung der Fische mit Calciumchlorid.

5. Diese Tatsachen weisen darauf hin, dass der Anpassung möglicherweise eine „Gerbung“ der Oberfläche des Tieres durch die höhere Konzentration der Salze des Seewassers, besonders des Calciums, zugrunde liegt.

THE APPLICABILITY OF THE PHOTOCHEMICAL ENERGY-LAW TO LIGHT REACTIONS IN ANIMALS.

By WOLFGANG F. EWALD.

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New York.)*

It has been pointed out by Loeb that tropistic light reactions in animals should follow the law of Bunsen and Roscoe. This law states that in a light reaction the effect is proportional to the simple product of intensity and time. It was first proved to be true for the formation of hydrochloric acid from chlorine and hydrogen and for the blackening of silver chloride under the influence of light. Later it was found to apply to the phototropic curvature (Fröschel, Blaauw) of plants, as well as to the human eye, though within rather narrow limits (Bloch, Charpentier). For light reactions in animals it has frequently been stated that they do not follow this simple law. A large number of forms seem to react to changes of intensity only, the effect in this case being proportional to the amount of change per unit of time. This is particularly true of the stimulating and inhibitory reflexes of the locomotor apparatus, as shown by a large number of investigators.

It occurred to me that it might be possible to get proof for the applicability of the energy-law by using a reaction which did not involve the locomotor organs. The eye movements of *Daphnia* seemed to afford a suitable object for the study of this question. These movements were first observed by Radl and his observations were confirmed and extended by myself some years later. The spherical eyeball containing a number of radially arranged ocelli is capable of rotation and held in position by several thin muscles inserted at its periphery. The eye shows a definite normal position with regard to light, a certain axis of the sphere having to be placed in such a direction that the ocelli on all sides of this axis get an

equal amount of illumination. The muscles keep the eye in this position and one can cause rotating movements of the eyeball, by shifting the position either of the source of light or of the animal. The eye will always maintain its fixed position to the source of light, no matter whether the body of the animal follows the eye or not. An unequal state of tension of the eye muscles seems to cause locomotor movements, which tend to restore the normal relative position of eye and body. By fixing the animal on a slide it can be prevented from moving and the eye movements may be observed at leisure. Instead of shifting the position of the light the eye can be placed in a position of equilibrium between two sources of light and eye movements can be caused by increasing or decreasing the intensity of either of them. This shows these movements to be a function of the intensity of illumination.

In order to test the energy-law, it is necessary to combine different light intensities with different times of exposure. If the product of time and intensity, *i. e.*, the amount of radiant energy brought to bear on the eye, is the same, the eye will always give the same reaction. To this end I proceeded in the following manner. The animal was fixed in a definite position on the stage of a microscope, illuminated from below by a weak electric light of constant intensity. The microscope stood in a blackened dark-room. Through a hole in the wall of the room the light of an 80 candle-power Tungsten lamp fastened outside could enter. The light was made diffuse by a sheet of oiled paper fixed across the opening. The hole was 55 mm. in diameter and was closed by a piece of cardboard containing two diaphragms of varying sizes, side by side. A shutter with a spring motion could alternately close either the one or the other opening. I could thus make an instantaneous change from a stronger to a weaker light, and *vice versa*, by using diaphragms of different sizes and moving the shutter to and fro. One diaphragm was maintained at constant size (25 mm. diameter) and a sector wheel or episcotister, driven by a small electromotor, could be rotated before it. The light passing this diaphragm had an intensity of 0.9 c.p. The distance between the animal and the diaphragm was about 60 cm. Obviously, if two diaphragms were used whose areas were as 1:10 and a sector wheel with 1/10 of the periphery cut out were

rotated before the larger one, so as to let light pass during $1/10$ of a revolution, then equal amounts of radiant energy would reach the eye of the animal through either diaphragm.

The microscope was placed in such a position that the light from the diaphragms could fall on the stage from the side. If the smaller diaphragm was opened, the eye of the *Daphnia* took up a position, defined by the ratio of intensities of the light coming from the weak lamp below and from the diaphragm above. Changing from the smaller to the larger diaphragm would cause a change in the position of the eye. By varying the sizes of the diaphragms I found that a noticeable reaction was obtained upon changing from one diaphragm to the other, even when the difference between their areas was as small as 10 per cent. Change between diaphragms of equal size, however, did not produce a reaction.

Using the diaphragm ratios 5:10, 2.5:10 and 1:10 I invariably found that upon using a sector wheel cutting down the time of exposure for the larger diaphragm so as to make the amount of energy equal to the smaller one, I obtained *no reaction* on change from one to the other. If I used sector wheels giving too long or too short exposures, a reaction was noticed, where the error exceeded 10 per cent. *These observations prove that for the eye movements of Daphnia the energy-law holds within the limits of accuracy characteristic of the reaction.* The speed of the sector wheel in these experiments was about $1/30$ of a second for one revolution. If slower speeds were used, marked deviations from the law began to appear, the intermittent having a weaker effect than the constant light. In some cases I got a marked reaction of the eye on change from constant to intermittent light of equal energy when the speed of the sector wheel was about $1/10$ of a second per revolution. The deviation becomes more marked, the slower the speed. The explanation for this phenomenon will be dwelt upon in a subsequent paper.

Strictly speaking, the law proved by my experiments is not the Bunsen-Roscoe law, but the law discovered more than twenty years earlier (1834) by Talbot, which states that the effect of intermittent light equals that of a constant light, if it emits the same amount of energy through a given period. In our case it means practically

the same as Bunsen-Roscoe's law, each revolution of the sector wheel constituting one period, in which there is a given relation between intensity and duration of the light flash and a definite time for reaction. The variously arranged sector wheels provide the possibility of testing different ratios. The constant light coming from the smaller diaphragm is used in such a way as to serve as a measure or standard of comparison and circumvent the necessity of determining a threshold of stimulation.

FURTHER EXPERIMENTS ON NATURAL DEATH AND PROLONGATION OF LIFE IN THE EGG.¹

By JACQUES LOEB.

(From the Laboratories of The Rockefeller Institute for Medical Research, New York.)

1. The unfertilized egg dies in a comparatively short time, while the act of fertilization gives rise to a theoretically at least unlimited number of generations. The death of the unfertilized egg may be called a case of "natural" death of a cell; it is perhaps the only case in which we can feel sure that death is caused by internal "natural" causes and not by avoidable injuries. The act of fertilization is the only one known to prevent natural death.

The velocity with which the unfertilized eggs die differs for the eggs of various species; the mature egg of the starfish dies much more rapidly than the egg of the sea urchin. The writer pointed out that this difference might be connected with a difference in the rate of oxidations in the two kinds of eggs, since he had been able to show that the suppression of oxidations by the withdrawal of oxygen from the sea water or by the addition of a trace of KCN prolongs the life of these eggs. Last year, Loeb and Wasteneys were indeed able to prove a difference in the relative rate of oxidations between the eggs of these two kinds of animals in the sense which is demanded by our hypothesis. The mature unfertilized egg of the starfish has a rate of oxidations which equals that of the fertilized egg;² while the rate of oxidations in the unfertilized mature egg of the sea urchin is only about one-fourth or one-sixth of that of the fertilized egg of the same species.

2. Our analysis of the process of the causation of development has led us to the result that it requires generally the coöperation

¹ Loeb, Maturation, natural death and the prolongation of life, etc. Biol. Bull., vol. 3, p. 295, 1902. The mechanistic conception of life, 1912.

² Loeb and Wasteneys, Arch. f. Entwicklungsmechanik, vol. 35, p. 555, 1912.

of two factors (or substances); one which calls forth the membrane formation (or the change in the cortical layer of the egg); and a second factor which saves the egg from the disintegration with which it is usually threatened after membrane formation. This second—corrective—factor is most conveniently supplied in the form of a short treatment of the egg with a hypertonic solution.³ The question therefore presented itself to which of these two factors the life-saving effect of the act of fertilization was due.

The natural inference from our former experiences would have been to ascribe the life-saving effect of the act of fertilization to the second corrective factor for the following reason. If we cause artificial membrane formation in the unfertilized egg of the sea urchin, we do not prolong its life but on the contrary we shorten it. Such an egg dies at room temperature in a few hours while without the membrane formation it may live at least for a few days. The artificial membrane formation alone (if it is not followed by the second corrective factor) hastens the death of the unfertilized egg. We can understand the reason for this since the artificial membrane formation accelerates the rate of oxidations in the sea urchin egg to exactly the same amount as the entrance of a spermatozoon (Warburg,⁴ Loeb and Wasteneys⁵). By the way of exclusion this seemed to restrict the life-saving effect of the act of fertilization as well as of artificial parthenogenesis to the second corrective factor.

3. Observations which the writer made this winter, however, show that this conclusion is not correct. In his earlier work he had already found that the hypertonic solution is just as effective as a corrective factor if it precedes the artificial membrane formation as if it follows it. The only difference between the two cases is a difference in the time of exposure required. When the artificial membrane formation is called forth first and the eggs are exposed to the hypertonic solution (50 cc. sea water + 8 cc. 2.5 m NaCl) afterwards, the eggs of *purpuratus* need remain in the solution for only from forty to sixty minutes. If, however, the

³ Loeb, *Die chemische Entwicklungserregung des tierischen Eies*, Berlin, 1909. The mechanistic conception of life, 1912.

⁴ Warburg, *Zeitschr. physiol. Chemie*, vol. 66, p. 305, 1910.

⁵ Loeb and Wasteneys, *Journ. Biol. Chemistry*, vol. 14, p. 469, 1913.

eggs are put into the hypertonic solution first and submitted to the treatment for membrane formation (e.g., butyric acid treatment) afterwards, they must remain in the hypertonic solution from 90 to 150 minutes. In the case of the egg of *Arbacia* this treatment in itself would induce artificial parthenogenesis, but in the egg of *Strongylocentrotus purpuratus* it does in most cases leave the eggs either intact or causes them to segment once or a few times and then to go into a state of rest again. When such eggs are afterwards treated with butyric acid they will develop.

The reason why the eggs must remain longer in the hypertonic solution when this treatment precedes the artificial membrane formation, than when it follows, seems clear if we consider the fact that the corrective effect of the hypertonic solution is weakened or inhibited if we inhibit or diminish the oxidations in the egg. This indicates that the corrective effect is in some way connected with the formation of a product of oxidation in the egg which is not formed in normal sea water. Since the rate of oxidations is from four to six times as great in the egg after the membrane formation as it is before, we can understand why the hypertonic solution brings about the corrective effect so much more quickly in the egg after membrane formation than before.

4. Working on the idea that the corrective effect of the hypertonic solution was due to the formation of a specific oxidation product in the egg it occurred to the writer to test whether the corrective effect of the hypertonic solution was reversible or permanent. Unfertilized eggs of *S. purpuratus* in which no membrane formation had been called forth were treated with a hypertonic solution and then portions of these eggs were treated after varying intervals of from one hour to three days with butyric acid. In all cases the butyric acid treatment now sufficed to call forth in these eggs a normal development at any time. This shows that the corrective effect produced by the hypertonic solution is irreversible and lasts in the egg as long as the latter lives.

The description of an experiment will illustrate this point. The unfertilized eggs of one female *S. purpuratus* were put for $\frac{1}{2}$, 1, $1\frac{1}{2}$, 2, $2\frac{1}{2}$, and $3\frac{1}{4}$ hours into hypertonic sea water (50 cc. sea water + 9 cc. 2.5 m NaCl + KCl + CaCl_2). None of these eggs devel-

oped into larvae and none of the eggs that had been up to $1\frac{1}{2}$ hours in the hypertonic solution segmented. Of the eggs that had been 2 hours in the hypertonic solution 0.5 per cent went into the two-cell stage but did not develop beyond this; of the eggs that had been $2\frac{1}{2}$ and $3\frac{1}{4}$ hours in the hypertonic solution 10 per cent segmented into the two- or as far as the four-cell stage. Practically all the eggs were intact the next day.

Twenty-two hours later part of each lot of these eggs was treated $1\frac{1}{2}$ or 3 minutes with propionic acid (2.8 cc. $\frac{N}{10}$ acid to 50 cc. sea water) to induce membrane formation. All formed tight fitting membranes. The result was as follows. The eggs that had been treated with the hypertonic solution $\frac{1}{2}$ and $1\frac{1}{2}$ hours disintegrated after the artificial membrane formation. The exposure to the hypertonic solution had been too short to produce a corrective effect. Among those treated for $1\frac{1}{2}$ hours one swimming larva was found the next day. Of those that had been in the hypertonic solution for 2 hours, 25 per cent segmented normally after the artificial membrane formation and developed into perfect blastulae; of those treated with hypertonic solution for $2\frac{1}{2}$ and $3\frac{1}{4}$ hours practically all developed but many of the blastulae were sickly, a sign that the eggs had been a little too long in the hypertonic solution.

Forty-eight hours after the treatment with hypertonic solution a second portion of the eggs that had been exposed $2\frac{1}{2}$ hours to the hypertonic solution was caused to form membranes by the treatment with propionic acid. The eggs had a tendency to stick to the glass but all segmented regularly and developed into blastulae which appeared normal.

Three days after the treatment of the eggs with the hypertonic solution another lot (those that had been two hours in the hypertonic solution) was treated with propionic acid. Those eggs that were still alive formed tight fitting membranes and all began to segment regularly. A few developed into normal blastulae.

On the fourth day all the eggs which had not been treated at all or had only been treated with the hypertonic solution were dead. Those eggs that had been treated with both the hypertonic and the propionic acid were developing and alive.

This experiment was repeated quite often with the same result.

It proves that the corrective effect of the hypertonic solution is irreversible and remains in the egg as long as it lives.

5. One word should be said in regard to the fact that the exposure of the eggs to hypertonic sea water which leads in *Arbacia* quite frequently to the origin of larvae, behaves so differently in the eggs of *S. purpuratus*. The writer has repeatedly pointed out this difference. His recent investigations have led him to think that this is due to a typical difference in the reactions of the eggs of the two species to the influence of the hypertonic solution. In both species the eggs of only part of the females can be induced to develop by the treatment with the hypertonic solution alone, but the number of females with susceptible eggs is much greater in *Arbacia* than in *S. purpuratus*. This year the writer has investigated what constitutes this difference in the behavior of the eggs of different females of *purpuratus* and found the following fact. The eggs of different females were treated with the same hypertonic solution at the same temperature and for the same time. The eggs of the majority of the females behaved as described in the preceding chapter of this paper. The eggs of some of the females of *purpuratus* developed into larvae through the mere treatment with a hypertonic solution. The difference between the two groups was this. The eggs which developed formed somewhat atypical membranes (gelatinous films), those that did not develop formed no membranes. The membrane formation did not necessarily occur while the eggs were in the hypertonic solution but often considerably later, while the eggs were already beginning to segment. Those eggs which had begun to segment but did not form the atypical membrane stopped developing and went back into the resting stage again. Those which formed membranes went on developing into larvae. The specific difference between the eggs of different females is therefore a difference in the facility with which the hypertonic solution causes membrane formation. The fatty acids, if properly applied, cause a typical or an atypical membrane formation in practically every sea urchin egg. The hypertonic sea water, however, causes an atypical membrane formation in the eggs of only a small percentage of the females of *purpuratus* and a larger though limited percentage of the eggs of *Arbacia*. As the writer

pointed out years ago, the purely osmotic method of artificial parthenogenesis gives better results if the solution is rendered more alkaline.

6. In order to avoid misunderstandings it may be well to point out that in the purely osmotic method of artificial parthenogenesis the causation of development is also due to two factors: a membrane-forming and a corrective factor. The peculiar fact is that one and the same external agency, the hypertonic solution, produces both effects simultaneously. It produces the corrective effect in all cases, if the eggs are left long enough in the solution. It induces membrane formation in a limited number of cases. The eggs develop into larvae only if both effects are produced by the hypertonic solution, otherwise the membrane formation has to be induced by some other agency, for example, the addition of some alkali to the solution or the treatment of the eggs afterward with a fatty acid.

It may be that the membrane formation by the hypertonic solution is always the combined effect of the HO ions and the hypertonic solution, even in neutral hypertonic solutions, where the $C_{OH} = 10^{-7}N$. But this conception may be unnecessary.⁶

7. We are now in a position to answer the question from which we started as to which of the two factors of fertilization (the membrane-forming and the corrective one) was responsible for the saving of the life of the egg. The answer must be that it is the combined effect of both factors. Our experiments have shown that the corrective effect if once imparted is permanent. If the corrective factor alone were the life-saving factor, eggs treated with a hypertonic solution alone should live indefinitely, or at least much longer than the non-treated unfertilized eggs. This is, however, not the case. In all the experiments the unfertilized eggs of *purpuratus* treated with the hypertonic solution alone died as quickly as those not treated at all, that is, in three days or less, at

⁶ In a former paper the writer had suggested that the HO ions contained in the hypertonic solution might furnish the first factor; this conclusion was reached under the influence of the experiments on *purpuratus* where a neutral hypertonic solution as a rule never induces artificial parthenogenesis. In *Arbacia*, however, a neutral hypertonic solution does easily induce the formation of parthenogenetic larvae.

room temperature. *For these eggs the artificial membrane formation became a life-saving act*; while the artificial membrane formation had just the opposite effect in sea urchin eggs, to which the corrective effect was not imparted. This admits of only one conclusion, namely that the life-saving effect of the act of fertilization is not due to one but to the combined action of both factors of fertilization.

This harmonizes with the observations on the eggs of such forms as the starfish (*Asterina*) in which the artificial membrane formation by a fatty acid suffices to call forth development. Not all the eggs, however, will develop into larvae under the influence of artificial membrane formation alone, but only a small percentage. R. S. Lillie has shown that the percentage of eggs of *Asterias*, which will develop into larvae after artificial membrane formation by heating, can be increased by a subsequent suppression of oxidations in such eggs (which treatment as the writer has shown can also supply the second factor of fertilization). This proves that in the egg of the starfish fertilization is also induced by two factors, but that some of the eggs possess or can produce the second factor normally, while this factor can be produced in the majority of eggs only if they are kept without oxidations for some time. In the sea urchin eggs it is a rare exception if the egg contains the second factor normally, that is, if it can develop into a larva by the mere act of membrane formation. As a rule, the second factor has to be supplied either by a treatment with a hypertonic solution or by a prolonged suppression of oxidations. The mature eggs of the starfish die without exception if they are not fertilized and they die much more rapidly than the unfertilized eggs of the sea urchin; while the mere artificial membrane formation saves at least the life of a small percentage of the eggs of the starfish. All these facts harmonize with the view that both factors of fertilization are required for the life-saving effect of fertilization. A similar situation exists in the case of the eggs of some annelids.

SUMMARY OF RESULTS.

1. Since the unfertilized egg dies in a comparatively short time while the act of fertilization saves the life of the egg, it was pro-

posed to find out which of the two factors of fertilization is responsible for this effect, the membrane formation or the corrective effect (produced in artificial parthenogenesis by the hypertonic solution). In former experiments the writer had shown that the artificial membrane formation alone hastens the death of the unfertilized egg while the treatment of such eggs with a hypertonic solution saves the life of the egg. This would make it appear as if the second factor was solely responsible for the life-saving effect of the act of fertilization.

2. The writer had formerly shown that the treatment of the egg with a hypertonic solution may precede the artificial membrane formation. It is shown in this paper that if the unfertilized eggs of *purpuratus* have once been treated with a hypertonic solution which in itself will not induce development they will develop at any time after artificial membrane formation has been induced. If the second factor is once imparted to this egg it keeps it as long as it is alive. The hypertonic solution induces an irreversible change in the egg.

3. It is shown that the treatment with a hypertonic solution alone does not prolong the life of the unfertilized egg. Eggs treated in this way live no longer than unfertilized eggs which have not been treated at all. If in such eggs artificial membrane formation is induced they will live indefinitely. For eggs treated with a hypertonic solution the artificial membrane formation becomes a life-saving act.

4. It follows from this that for the prolongation of the life of the unfertilized egg both factors of artificial parthenogenesis, the alteration of the surface as well as the second factor (usually supplied by the treatment with hypertonic solution) are required.

FURTHER OBSERVATIONS ON ARTIFICIAL PARTHENOGENESIS IN FROGS.

BY JACQUES LOEB AND F. W. BANCROFT.

(*From the Laboratories of The Rockefeller Institute for Medical Research, New York.*)

1. We have repeated our efforts to raise parthenogenetic frogs during the past season in order to be able to determine their sex, but with less success than we had last year. None of the parthenogenetic tadpoles which we produced this year lived as long as those of the previous year.

During the past two seasons we have punctured the eggs of *Rana sphenoccephala*, *R. pipiens*, *R. silvatica*, *Chorophilus feriarum* and of *Bufo americanus*. Only the eggs of the first two species could be utilized for our purpose, inasmuch as it is possible in them alone to obtain swimming parthenogenetic tadpoles from the punctured eggs. If the eggs of the other species mentioned are punctured they may begin to segment but they do not reach the tadpole stage. The frogs were caught in the open while copulating, the sexes separated and sent immediately to the laboratory. Some of the frogs (*R. pipiens*) were obtained in Long Island and some were sent from Chicago. The results were in both cases the same, in spite of the fact that the eggs of the frogs sent from Chicago must have remained in the uterus several days. From 600 to 1000 eggs of each female were punctured. The results were similar to those described in our last paper,¹ except that this spring all the swimming parthenogenetic tadpoles died when they were from twenty to thirty days old. Some of the eggs were smeared with blood before they were punctured. While the percentage of the eggs which underwent early segmentation was possibly augmented by this procedure, the percentage of the eggs which developed into tadpoles was not increased in this way.

¹ Jour. Exp. Zool., vol. 14, p. 275.

Practically all the eggs of the same females that had been fertilized with sperm developed into tadpoles, many of which are healthy and alive today.

2. We were anxious to obtain parthenogenetic frogs in order to test the statement of Kuschakewitsch,² to which Professor Morgan called our attention, that the young frogs are often hermaphrodites, or intermediate forms. If Kuschakewitsch's statement is correct, the presence of ova in the genital glands is not sufficient proof that the young frog is going to develop into a female. He gives some criteria for the discrimination between real females and the intermediate form which may develop into males. On the basis of his statements we examined once more the sections of the sexual glands of last year's parthenogenetic frog. We also utilized the slides we had made from the sexual glands of a young frog produced by fertilization with sperm from an egg of the same female from which the parthenogenetic tadpole and frog originated.

The gonads of the parthenogenetic frog which died at the close of metamorphosis were fairly well preserved. An examination showed that there were eight or nine genital spaces. According to Kuschakewitsch eight genital spaces are characteristic for the intermediate form while six are characteristic for the pure female. In addition there were many degenerating ova, which Kuschakewitsch mentions as a characteristic for the intermediate form. Comparatively few of the oöcytes had entered the growth period. The right gonad, which was smaller and in an earlier developmental stage than the left one, had nineteen larger oöcytes while the left gonad had only fifteen. Most of the oöcyte nests (or oögemmae as Kuschakewitsch calls them) did not contain any large oöcytes. If Kuschakewitsch's criteria are correct, the parthenogenetic frog which we described is a hermaphrodite or intermediate.

According to Kuschakewitsch these intermediates may develop into either males or females, and there are indications in the sections of the parthenogenetic frog that this transformation may have been under way. In the anterior part of the left gonad of

² Festschr. f. Richard Hertwig, Bd. 2, p. 61.

the parthenogenetic frog the sections show all the appearances which Kuschakewitsch (p. 145) considers characteristic for the early stages of the transformation of the intermediate form into males. The endothelial lining of the secondary genital spaces is thicker than usual, more convoluted, and mitotic figures may be seen in places. In some regions considerable masses of undifferentiated embryonic tissue have been formed which are continuous with the endothelium of the secondary genital spaces and occupy almost the whole cross section of the gonad except for a few patches of germinative endothelium. If we may rely upon the statements of Kuschakewitsch, our frog would have developed into a male if it had survived.

The gonads of the parthenogenetic tadpole were so poorly fixed that nothing could be made out about them except that they contained many more large oöcytes than those of the frog. It could not be determined whether this individual was a female or belonged to the intermediate form.

The frog which had originated from a fertilized egg of the same female from which the parthenogenetic tadpole and frog were obtained, was killed about four months after metamorphosis. It had grown rapidly. The gonads were evidently those of a male. The spermatic follicles contained cells in all stages of spermatogenesis from spermatogonia to spermatids with greatly elongated nuclei. In addition almost every section showed follicles which were nearly filled with a large oöcyte in the early growth period. Thus it appears that this male may have developed from a hermaphrodite or intermediate, if Kuschakewitsch's statements are correct.

In the European species, *R. esculenta*, the formation of spermatozoa does not take place for several years after metamorphosis while in the control frog this process was well under way about four months after metamorphosis. It would seem, then, that in *Rana sphenoccephala*, which is a southern species, sexual differentiation and sexual maturity set in a little more rapidly than in *Rana esculenta*.

It is obvious that further observations are needed to determine definitely the sex of parthenogenetic frogs.

DIE BEDEUTUNG DES CALCIUMS FÜR DAS WACHSTUM.

VON FRANCIS H. MCCRUDDEN.

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MIT TAFEL I UND II.

Während der letzten 2 Jahre habe ich mich im Rockefeller Institute mit der Frage des Infantilismus beschäftigt. Das Interesse für dieses Problem wurde von einem durch Stoffwechselstörungen charakterisierten Typus angeregt. Die Störungen betrafen hauptsächlich den Darmkanal. Es war die Möglichkeit gegeben, dass chemische Studien in mancherlei Beziehung eine Lösung nicht nur für die Ätiologie dieses speziellen Typus, sondern auch für die Frage des Zwergwuchses im allgemeinen erbringen könnten. Die erhaltenen Resultate sind sehr interessant und bedeuten unzweifelhaft einen Fortschritt auf dem Gebiete der Wachstumsforschung.

EIWEISSSTOFFWECHSEL.

Vorläufige Studien zeigten, dass die Fäces verhältnismässig massenhaft waren und viel Stickstoff enthielten; der Harn dagegen stickstoffarm und bei Fleisch-freier Diät kreatinhaltig war. In einem Falle wurde gelegentlich Aceton, nie aber Glykose gefunden. Diese Befunde legten den Gedanken nahe, dass es sich um einen Hungerzustand irgendwelcher Art handelte. Um diesen Punkt aufzuklären wurde die Verteilung des Stickstoffs und des Schwefels im Harne studiert. Unter normalen Verhältnissen werden mehr als 80% des Gesamtstickstoffs als Harnstoffstickstoff, und ungefähr 80% des Gesamtschwefels als anorganischer Schwefel ausgeschieden. Die Ausscheidung anderer stickstoff- und schwefelhaltiger Substanzen ist niedrig. Während des Hungers oder bei einer niedrigen Eiweissdiät vermindert sich die prozentuelle Menge des Harnstoffs und der Sulfate; sie vergrössert sich dagegen für die Harnsäure, das Kreatinin, das Ammoniak, die Aminosäuren und

sowohl für die Ätherschwefelsäure als auch den neutralen Schwefel. Die Bestimmung der Ausscheidungsformen des Stickstoffs und des Schwefels würde uns in jedem einschlägigen Falle zeigen, welcher von den zwei Zuständen vorliegt. In unseren Fällen bildete der als Harnstoffstickstoff ausgeschiedene Stickstoff immer mehr als 80% des Gesamtstickstoffs; und der Sulfatschwefel ebenfalls mehr als 80% des Gesamtschwefels¹⁾. Die Resultate waren somit in jeder Hinsicht normal und ähnlich denjenigen, die man bei einer eiweissreichen Diät erwartet.

Häufige und langandauernde Darmstörungen in der Krankengeschichte, massenhafte, in weitgehender Fäulnis begriffene Stühle, bedeutender Verlust an Stickstoff mit den Fäces einerseits und Stickstoffarmut des Harnes andererseits sprachen immer in dem Masse für die Hypothese eines Hungerzustandes, dass trotz der scheinbar normalen Stickstoff- und Schwefelverteilung im Harn es mir doch ratsam schien, die Fälle einem gründlicheren Studium zu unterwerfen, bevor ich mich befähigt fühlte, die Hungerhypothese ganz auszuschliessen. Es bestand die Möglichkeit, dass die Nahrungseiweissstoffe im Magendarmkanal zu rasch oder zu weitgehend gespalten werden oder aber dass aus denselben schädliche oder unbrauchbare Produkte entstehen könnten und auch dass sie nur unvollständig hydrolysiert und absorbiert werden. Es wurden daher in den Fäces Bestimmungen vom Gesamtstickstoff und vom Stickstoff des gelösten Eiweisses, des Ammoniaks, der Purinverbindungen, der Bakterienkörper und der freien Aminosäuren gemacht und mit normalen Werten verglichen (7). Ferner wurden auch die Ätherschwefelsäuren des Harnes bestimmt. Aus den Daten ist es möglich Schlüsse zu ziehen mit Bezug auf die Eiweissabsorption, Eiweissverdauungsprodukte und die Intensität und Natur der Bakterientätigkeit in Zusammenhang mit den vorliegenden Problemen über die Hungerzustände. Ich fand, dass die Eiweisstoffe ebenso weitgehend zu absorptionsfähigen Substanzen abgebaut werden wie normalerweise. Es lag kein Grund vor, eine abnorme Tätigkeit der Darmflora anzunehmen weder nach dem Ammoniak- noch dem Bakterien- oder dem Purinstickstoff der Fäces, noch nach der Quantität der Ätherschwefelsäure des Harnes. Die Menge der nicht absorbierten freien Aminosäuren war ebenfalls

1) Literatur am Ende der Arbeit.

nicht abnorm verändert. Bei diesen Untersuchungen besteht also keine Abweichung vom Normalen. Die Stickstoffverteilung in den Fäces wurde normal gefunden. Die verschiedenen Anteile sind somit wahrscheinlich derselben Herkunft; namentlich sind sie hauptsächlich exkretorische Stoffe und nicht unresorbierte Nahrungsreste.

Die Frage nach der Natur des Stickstoffs in den Fäces in Beziehung zur Absorption wurde weiter durch längere Zeit andauernde Verabreichung einer eiweissreichen Diät untersucht. Jede Schwierigkeit in der Verdauung oder Resorption des Stickstoffmaterials würde sich natürlich unter diesen Umständen vergrössern und besser zum Vorschein kommen. Der Stickstoffgehalt der Fäces würde auch bedeutender werden. In der Tat aber fand ich bei einer 5 maligen Vermehrung des eingenommenen Stickstoffs den Stickstoffgehalt der Fäces nicht nur nicht vergrössert, sondern sogar vermindert (9).

KOHLLENHYDRAT- UND FETTSTOFFWECHSEL.

Dass der Organismus sich vielleicht doch in einem Hungerzustande anderer Art befand, dafür konnte man die Kreatin- und Acetonausscheidung in Betracht ziehen. Diese zwei Substanzen werden ausgeschieden, wenn der Körper die Fähigkeit Zucker zu oxydieren verloren hat oder wenn der Kohlenhydratvorrat erschöpft wird, wie es bei Diabetes und in Hungerversuchen der Fall ist. Es schien möglich, dass in solchen Fällen die Fähigkeit Glykogen zu verarbeiten oder anzuhäufen, verloren geht, so dass der Vorrat bald zu Ende kommt—ein Zustand von chronischem Kohlenhydrathunger. Um diese Hypothese zu erproben, wurde der respiratorische Quotient nach einem 18stündigen Hungern bestimmt. Durchschnittlich wurde eine Zahl von 0,79 gefunden (2). Dies bedeutet, dass die Kohlenhydrate und auch das Fett oxydiert waren und der Vorrat an Glykogen selbst nach 18stündigem Hungern nicht erschöpft wurde.

Die Fäces zeigten ganz normale Mengen von Totalfett, Fettsäuren und Seifen (8).

Versuche, die Acetonbildung als eine Folge von Acidosis zu erklären, gaben ebenfalls negative Resultate. Die Ammoniakaus-

scheidung betrug ein Viertel Gramm im Tage—ein normaler Wert (2).

Ein weiterer Beweis, dass die allgemeine Ernährung auf einem hohen Niveau steht, liegt in der Tatsache, dass mit der Nahrung verabreichtes Kreatin wieder im Harne erschien (9). Dies geschieht nur bei gut genährten Individuen¹⁾.

Die Untersuchung des Kraftstoffwechsels ergab, dass die kalorimetrisch gemessene Intensität des Ruhestoffwechsels pro Quadratmeter Körperoberfläche 0,775 Kalorien in 24 Stunden betrug, was ein normaler Wert ist (11).

In der Tat, alle Beweise lehren, dass keine Abnormität weder der Verdauung noch der Absorption und Verarbeitung sowohl der Proteine als auch der Fette und Kohlenhydrate der Nahrung besteht, dass der allgemeine Ernährungszustand der weichen Gewebe ein guter ist, dass der Kraftstoffwechsel normal ist, dass wir es also bei keinem der Zwergtypen, die ich studierte, mit einer Tendenz zum Hunger oder zu mangelhafter Ernährung im gewöhnlichen Sinne des Wortes zu tun haben.

DAS SKELETT UND DER ANORGANISCHE STOFFWECHSEL.

Im Laufe meiner Untersuchungen über den Zwergwuchs beobachtete ich einige Anomalien mit Bezug auf den Calciumstoffwechsel. Vorherige Studien über den anorganischen Stoffwechsel in Fällen von Osteomalacie waren so erfolgreich für die Erklärung der Pathogenese und der Ätiologie jener Krankheit (5. und 6), dass solche Untersuchungen auch an Zwergen unternommen wurden. Es wurden vollständige Bilanzstudien durch Bestimmungen von Stickstoff, Schwefel, Phosphaten, Calcium und Magnesium in den Nahrungsmitteln, dem Harne und den Fäces für Perioden von 6 bis 10 Tagen durchgeführt.

Tabelle I, II, IV, V und VI am Schlusse der Arbeit zeigen die Resultate an Zwergen von verschiedenen Typen. Tabelle III gibt die Resultate der Untersuchung an einem normalen Knaben als Vergleich wieder. Wichtig dabei ist, dass in den mit F. S. und F. H. bezeichneten Fällen, welche lange andauernde Darmstörungen

1) Jolin, The chemistry and biochemistry of kreatin and kreatinin. Festschrift f. O. Hammarsten, III, 1906.

in der Krankengeschichte zeigen, **das Calcium fast vollständig im Harn fehlt.** Die Werte sind absolut und relativ niedrig im Vergleich zu den anderen Fällen, zu den anderen Elementen und zum Calcium der Fäces. **Die Fäces dieser Fälle sind sehr reich an Calcium und die Calciumbilanz ist negativ.** Die anderen Zwerge zeigten einen normalen Calciumstoffwechsel.

Die Bilder auf Tafel I u. II stellen die zarten Knochen mit dünnem Cortex dar. Dieselben wurden speziell ausgewählt um das Peroneum, die Knochen des Metacarpus und Metatarsus der Zwerge F. S. und F. H. mit denjenigen des normalen Knaben und der anderen Zwerge zu vergleichen. Wichtig ist zu notieren, dass bei F. S. und F. H., den Fällen mit Störungen im Calciumstoffwechsel, der Knochencortex sehr zart und dünn ist. Bei den Fällen mit normalem Calciumstoffwechsel ist dagegen das Knochengewebe von normalem Charakter. Die Differenzen sind nicht der Technik zuzuschreiben; es wurden mehrere Aufnahmen mit demselben Resultate gemacht.

Man kann einwenden, dass die negative Calciumbilanz nicht dauernd besteht. Vielleicht tut sie es nicht. In dieser Beziehung kann der Fall wie bei der Osteomalacie sein, bei welcher in der akuten Periode eine negative Calciumbilanz beobachtet wird. Die negative Bilanz nimmt mit dem Fortschritte der Krankheit immer ab, bis endlich in den terminalen Phasen der schweren Fälle der Organismus sein letztes unentbehrliches Calciumquantum hartnäckig zurückhält und ein Gleichgewicht niedrigeren Niveaus allmählich erreicht wird (5).

Es gibt ferner noch andere Gründe für die Annahme, dass das Zurückbleiben im Wachstum eher speziell mit einer Stoffwechselstörung des Calciums als mit einem Zustande von allgemeiner mangelhafter Ernährung verbunden ist. Infolge einer sorgfältigen diätetischen Behandlung nach dem ersten Stoffwechselversuche verbesserte sich der allgemeine Status und Ernährungszustand bei F. S. wesentlich. Aus dem mageren, schwachen, elend aussehenden bettlägerigen Kinde wurde in einigen Monaten ein gutaussehender, fetter, lustiger Knabe, der im Krankenhause herumlief. Trotz der Verbesserung im allgemeinen Zustande versagten jedoch die Kräfte eines normalen Wachstums und die Knochen blieben zart und dünn.

Die folgenden Zahlen zeigen die Resultate der Harnuntersuchung nach der Verbesserung (10):

	Stickstoff	Calcium- oxyd	Magnesium- oxyd	Phosphor- pentoxyd	Schwefel
10 tägige Periode	54,85	0,198	2,186	14,03	3,64
Durchschnitt pro die	5,49	0,0198	0,2186	1,40	0,364

Gleichzeitig mit der Besserung im Ernährungszustande der weichen Gewebe wurde eine Erhöhung des Stoffwechsellniveaus der Elemente (Stickstoff, Magnesium, Phosphor und Schwefel) dieser Gewebe beobachtet. Der Calciumstoffwechsel blieb jedoch abnorm.

Ich fahndete nachher nach der Ursache des Calciumverlustes durch die Fäces (8). Derselbe ist nicht mit einer vermehrten Ausscheidung von Phosphaten oder mit einer abnorm reichen Bildung von Fett- und flüchtigen Säuren verbunden. Die Phosphatausscheidung ist normal; die Menge der Fett- und flüchtigen Säuren klein und das Verhältnis der Fettsäuren und Seifen zum Totalfett normal.

DISKUSSION.

Wir können die Fälle von Zurückbleiben im Wachstum in 2 Klasseneinteilen. Die eine (F.S., F.H.) zeigt eine Störung im Calciumstoffwechsel. Dieses Element verschwindet fast aus dem Harne und wird dagegen in grossen Mengen mit den Fäces ausgeschieden. Da kein Beweis irgendeines krankhaften Zustandes der Niere vorliegt, der den Übergang des Calciums in den Harn verhindern könnte, so muss entweder das Calcium des Blutes in einer eigentümlichen unausscheidbaren und vom Knochen unverwertbaren Verbindung existieren, oder es muss das Blut sehr arm an Calcium sein. Letztere Hypothese ist wahrscheinlicher und kann durch den grossen Verlust an Calcium durch die Fäces erklärt werden. Das Blut muss sehr arm an Calcium sein. Der klinische Befund dieses Typus ist eine Unterentwicklung und Zartheit der Knochen. Die andere Klasse (J. P., E. B., M. S.) ist nicht mit einer Störung des Calciumstoffwechsels verbunden und die Knochen sind normal an Dicke und Resistenz.

Nach Rubner¹⁾ haben wir im Wachstumsprozess zwischen

1) Rubner, Arch. f. Hyg. 1908, XVI, 81.

dem "Wachstumstrieb," welcher im jungen Organismus vorhanden ist und im alten fehlt, und dem Wachstumsmaterial zu unterscheiden. Beide Faktoren sind für ein normales Wachstum notwendig. In unseren zwei Typen des Zwergwuchses können wir Störungen dieser beiden Faktoren erkennen. Beim Typus mit dünnen Knochen und Erniedrigung der Blutcalciummenge hängt die Unfähigkeit zum Wachsen vom Fehlen eines genügenden für das Wachstum des Skeletts zur Verfügung stehenden Calciumquantums ab. Die fundamentale Tendenz zum Wachstum scheint nicht zu fehlen. Die weichen Gewebe wachsen so rasch wie das Skelett und das Skelett wächst so rasch als das anwesende Material (Calciumsalze) es erlaubt; rascher, in der Tat, als die Quantität es erlauben würde, wenn sich daraus normalstarke Knochen bildeten. Im zweiten Typus gibt es kein Fehlen an Wachstumsmaterial. Bei diesem Typus scheint die Ursache mehr in der Abwesenheit von dem, was als Wachstumstendenz bezeichnet wird, zu liegen.

SCHLUSSFOLGERUNG.

Kurz zusammengefasst gibt es in manchen Fällen von Zurückbleiben im Wachstum eine mangelhafte Entwicklung des Skeletts und Störungen des Calciumstoffwechsels. Die Knochen sind zart und leicht brüchig. Es werden grosse Mengen Calcium durch die Fäces verloren. Der Harn ist fast frei von diesem Metall. Es ist wahrscheinlich, dass das Zurückbleiben in der Entwicklung des Skeletts in diesen Fällen auf einer Abwesenheit der zum Wachstum der Knochengewebe zur Verfügung stehenden Calciumsalze beruht. Andere Formen des Zwergwuchses zeigen keine Störungen des Calciumstoffwechsels und das Knochengewebe ist von normaler Solidität. In diesen Fällen haben wir es eher mit einer mehr fundamentalen Abwesenheit des "Wachstumstrieb" als mit einem Mangel an Wachstumsmaterial zu tun.

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TABELLE I.

Knabe F. S. (Infantilismus). Gewicht 14,3 Kilo, Alter 11 Jahre.

Tag	Gewicht der Fäces	Stickstoff	Calcium- oxyd	Magnesium- oxyd	Phosphor- pentoxyd	
Harn	1	29,6	2,75	0,010	0,005	0,512
	2		2,76	0,016	0,070	0,520
	3		3,02	0,021	0,086	0,572
	4		2,75	0,012	0,074	0,497
	5		2,51	0,009	0,054	0,398
	6		2,32	0,040	0,111	0,211
Total		16,11	0,108	0,400	2,710	
Fäces	1	29,6	1,707	1,965	0,213	0,855
	2	14,32	0,868	0,750	0,009	0,767
	3	58,5	3,532	2,744	0,579	2,495
	4	40,35	2,840	1,733	0,339	1,077
	5	52,55	2,286	1,618	0,331	1,072
	6	35,75	2,288	1,451	0,229	1,073
Total		231,1	13,52	10,26	1,700	7,34
Gesamtausscheidung		29,63	10,37	2,10	10,25	
Gesamteingabe		35,64	6,35	2,43	11,91	
Retiniert		6,01	—	0,33	1,66	
Verlust		—	4,02	—	—	
Prozent retiniert		16,9	—	1,4	13,9	
Prozent verloren		—	63,3	—	—	

TABELLE II.

Knabe F. H. (Infantilismus). Gewicht 12,0 Kilo, Alter 5½ Jahre.

Tag	Gewicht der Fäces	Stickstoff	Schwefel	Calcium- oxyd	Magnesium- oxyd
Harn	1	5,68	0,432	0,050	0,061
	2	5,44	0,412	0,017	0,058
	3	3,27	0,392	0,014	0,063
	4	5,62	0,428	0,028	0,068
	5	5,87	0,452	0,018	0,073
	6	5,56	0,424	0,029	0,067
Total		33,44	2,540	0,156	0,390
Fäces	1	17,4	0,789	1,583	0,300
	2	21,3	0,989	1,632	0,337
	3	17,5	0,836	1,279	0,287
	4	18,7	0,899	1,265	0,308
	5	22,7	1,016	1,577	0,336
	6	19,7	0,875	1,449	0,274
Total	117,3	5,404	0,675	8,785	1,842
Gesamtausscheidung		38,84	3,216	8,941	2,231
Gesamteingabe		48,77	4,110	8,820	1,968
Retiniert		9,93	0,894	—	—
Verloren		—	—	0,121	0,263
Prozent retiniert		20,3	21,8	—	—
Prozent verloren		—	—	1,4	1,3

TABELLE III.

W. McC., normaler Knabe (als Vergleich). Gewicht 27,2 Kilo, Alter 14 Jahre.

Tag	Gewicht der Fäces	Stickstoff	Schwefel	Calcium- oxyd	Magne- siumoxyd	Phosphor- pentoxyd
Harn	1	10,43	0,729	0,374	0,153	2,34
	2	10,65	0,744	0,406	0,139	2,22
	3	10,35	0,717	0,373	0,154	2,23
	4	11,07	0,832	0,346	0,152	2,06
	5	11,35	0,808	0,332	0,157	2,58
	6	11,12	0,810	0,390	0,154	2,68
Total		64,97	4,640	2,223	0,909	14,11
Fäces	1	22,8	1,232	1,518	0,267	0,686
	2	19,0	0,997	1,846	0,263	0,718
	3	20,9	1,165	1,784	0,288	0,768
	4	23,5	1,173	2,084	0,301	0,839
	5	15,3	0,811	1,318	0,220	0,526
	6	21,1	1,110	1,643	0,279	0,856
Total	122,6	6,488	0,582	10,19	1,618	4,393
Gesamtausscheidung		71,46	5,222	12,42	2,53	18,50
Gesamteingabe		86,10	6,138	14,99	3,263	24,71
Retiniert		14,64	0,916	2,57	0,73	6,21
Prozent retiniert		17,0	14,9	17,1	22,4	25,0

TABELLE IV.

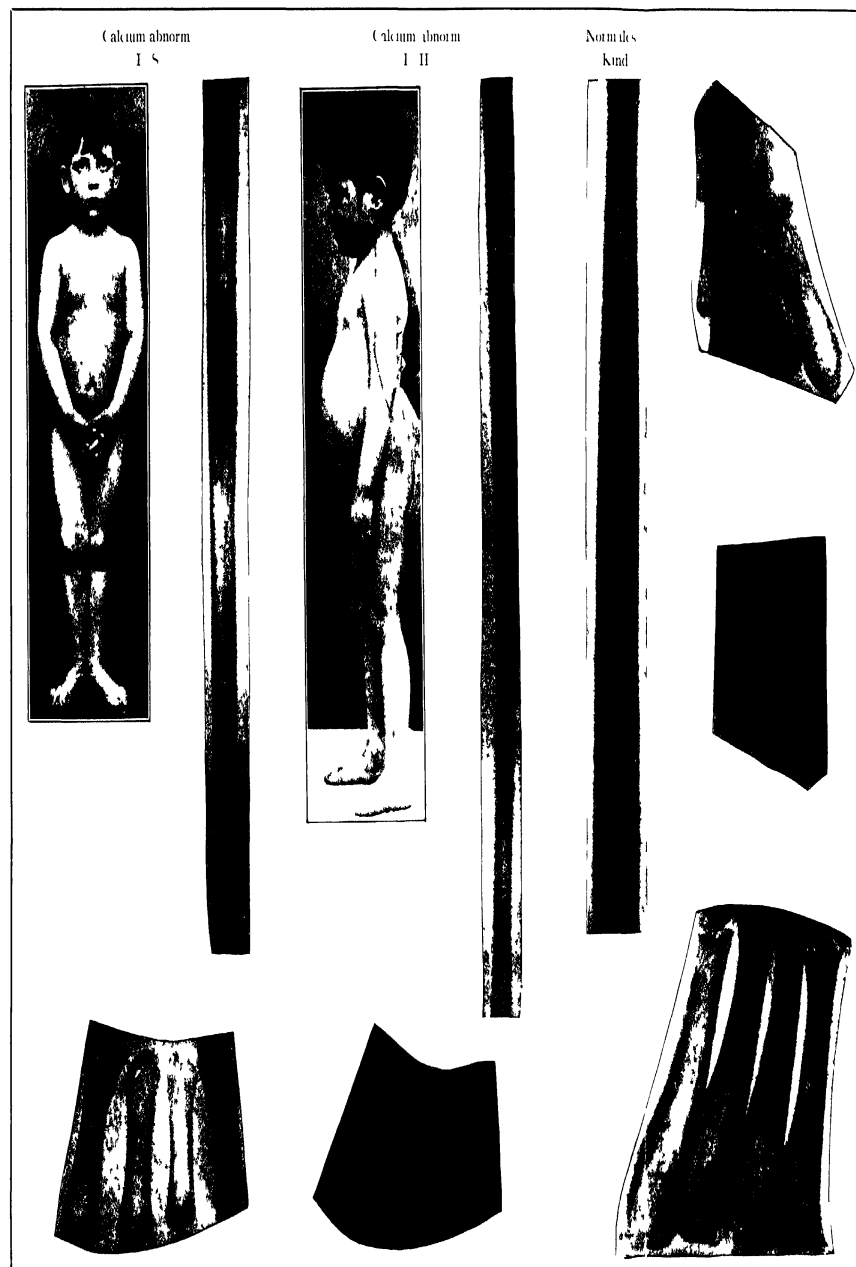
J. P., Zwerg des Lorraine'schen Typus. Gewicht 22,0 Kilo, Alter 17 Jahre.

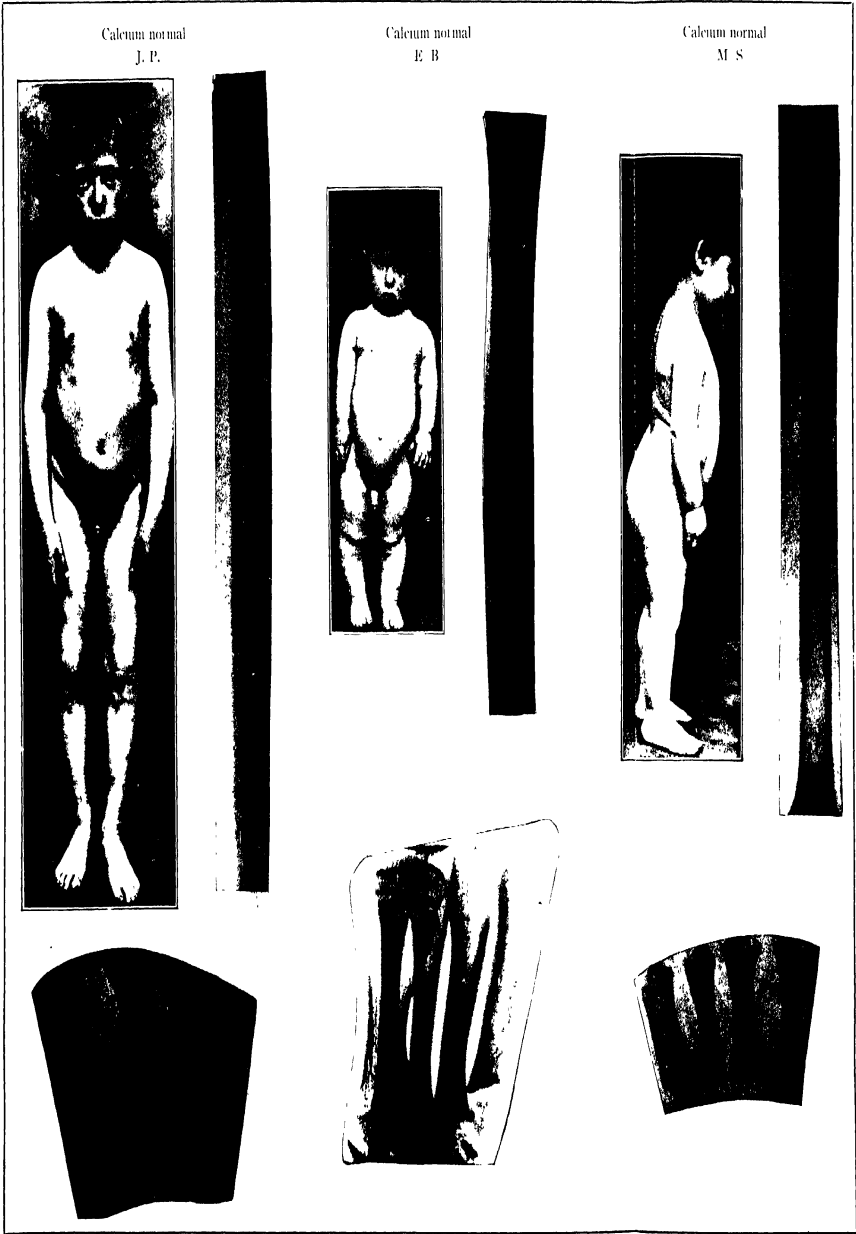
Tag	Gewicht der Fäces	Stickstoff	Schwefel	Calcium- oxyd	Ma- gnesium- oxyd	Phosphor- pentoxyd
Harn	1	5,50	0,340	0,0985	0,1180	1,285
	2	6,94	0,480	0,1086	0,1030	1,354
	3	7,40	0,488	0,1250	0,1130	1,433
	4	7,16	0,484	0,1333	0,1100	1,164
	5	6,56	0,448	0,0950	0,0945	1,212
	6	7,24	0,492	0,1165	0,1095	1,284
	7	6,58	0,424	0,1325	0,1190	1,315
	8	6,04	0,404	0,1135	0,1114	1,208
	9	6,82	0,480	0,1175	0,1015	1,284
	10	6,39	0,416	0,1225	0,1165	1,004
Total		66,62	4,456	1,163	1,088	12,54
Fäces	1	12,80	0,561	0,0513	0,129	0,434
	2	16,04	0,962	0,0883	0,128	0,210
	3	12,75	0,818	0,0778	0,584	0,334
	4	15,32	0,964	0,0997	0,645	0,305
	5	15,93	0,038	0,0829	0,630	0,365
	6	13,43	0,077	0,0725	0,498	0,231
	7	16,79	0,928	0,0838	0,805	0,273
	8	15,95	0,823	0,0793	0,572	0,111
	9	16,60	0,818	0,0882	0,492	0,146
	10	21,36	1,170	0,1347	0,844	0,149
Total		8,759	0,8585	5,931	1,466	3,042
Gesamtausscheidung		75,38	5,315	7,094	2,554	15,585
Gesamteingabe		86,42	6,700	9,436	3,251	19,210
Retiniert		11,04	1,385	2,342	0,707	3,625
Prozent retiniert		12,8	20,7	24,8	21,7	18,9

TABELLE V.

E. B., Achondroplastischer Zwerg. Gewicht 13,7 Kilo, Alter 4 Jahre.

Tag	Gewicht der Fäces	Stickstoff	Schwefel	Calcium- oxyd	Ma- gnesium- oxyd	Phosphor- pentoxyd
Harn	1	5,419	0,387	0,143	0,100	1,272
	2	5,102	0,374	0,124	0,095	1,183
	3	4,867	0,350	0,130	0,109	1,058
	4	4,836	0,340	0,130	0,103	1,056
	5	4,362	0,348	0,112	0,100	1,114
	6	5,220	0,408	0,149	0,115	1,272
	7	5,059	0,355	0,131	0,109	1,138
	8	5,149	0,375	0,141	0,114	1,079
Total		40,01	2,937	1,06	0,845	9,172





(Fortsetzung von Tabelle V.)

Tag	Gewicht der Fäces	Stickstoff	Schwefel	Calcium- oxyd	Ma- gnesium- oxyd	Phosphor- pentoxyd	
Fäces	1	18,89	0,980	0,109	0,614	0,217	0,610
	2	12,21	0,650	0,071	0,419	0,136	0,448
	3	24,91	1,407	0,152	0,765	0,306	0,842
	4	20,51	1,193	0,115	0,628	0,242	0,665
	5	14,91	0,835	0,079	0,432	0,194	0,516
	6	14,28	0,776	0,074	0,558	0,158	0,548
	7	19,27	0,995	0,111	0,802	0,212	0,636
	8	35,38	1,812	0,206	0,976	0,435	1,338
Total		8,648	0,917	5,194	1,900	5,603	
Gesamtausscheidung		48,66	3,854	6,254	2,745	14,78	
Gesamteingabe		54,44	4,221	8,217	3,056	18,29	
Retiniert		5,78	0,367	1,963	0,311	3,51	
Prozent retiniert		10,6	8,7	23,9	10,2	19,2	

TABELLE VI.

M. S., Kretin. Gewicht 17,8 Kilo, Alter 8 Jahre.

Tag	Gewicht der Fäces	Stickstoff	Schwefel	Calcium- oxyd	Ma- gnesium- oxyd ¹⁾	Phosphor- pentoxyd	
Harn	1	6,336	0,442	0,129	0,0340	1,248	
	2	5,572	0,382	0,114	0,0384	1,225	
	3	6,475	0,515	0,120	0,0240	1,222	
	4	4,938	0,294	0,091	0,0297	0,897	
	5	5,177	0,376	0,144	0,0382	0,917	
	6	5,173	0,412	0,137	0,0339	0,923	
	7	5,014	0,364	0,116	0,0275	0,936	
	8	4,958	0,348	0,128	0,0338	0,954	
Total		43,64	3,133	0,979	0,2595	8,322	
Fäces	1	6,6	0,347	0,0508	0,568	0,128	0,533
	2	8,4	0,504	0,0648	0,600	0,141	0,533
	3	6,1	0,373	0,0398	0,403	0,116	0,327
	4	7,0	0,434	0,0513	0,441	0,139	0,381
	5	8,1	0,457	0,0614	0,536	0,157	0,460
	6						
	7	10,4	0,539	0,0521	0,693	0,182	0,630
	8	10,65	0,589	0,0573	0,600	0,190	0,614
Total		3,243	0,3775	3,841	1,053	3,478	
Gesamtausscheidung		46,89	3,511	4,820	1,313	11,80	
Gesamteingabe		57,32	3,573	6,996	1,481	16,19	
Retiniert		10,43	0,062	2,176	0,168	4,39	
Prozent retiniert		18,1	1,74	31,10	11,34	27,14	

¹⁾ Merkwürdigerweise ist die Magnesiumausscheidung mit dem Harn sehr niedrig.

A CASE OF AURICULAR FIBRILLATION WITH A POST MORTEM EXAMINATION.*

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CASE REPORT.

History.—The patient, Mrs. T. G., was 37 years old. Her weight was 55 kilos. She was first admitted to St. Francis Hospital, Pittsburgh, Jan. 17, 1912; her chief complaint being shortness of breath. Dyspnea had been present during the past four to six weeks, during which period she had also experienced palpitation and precordial pain; occasional night sweats had occurred during the past few months, together with frequent "colds," during the course of which the patient would bring up blood-stained sputum. Her past medical history was as follows: She had chorea during childhood, rheumatic fever in 1908 and again in 1911, measles, diphtheria and small-pox in early life. She was not subject to sore throat. The patient had given birth to three healthy children and had had no miscarriages. She had worked hard. Her habits were good.

Examination.—The physical findings on the day following admission were, in brief, as follows: The patient was a slender, middle-aged woman, lying in bed without any apparent pain or discomfort. Her temperature was 98.4 F., pulse-rate 120 to 130, respiration 22 to 24, systolic blood-pressure 120. She coughed occasionally. There was slight cyanosis, but no jaundice nor edema. Her eyes, mouth and tonsils were negative. The external jugular veins were pulsating. The thyroid gland was not enlarged. The thorax was barrel-shaped and allowed only shallow respiratory excursions; there was a pronounced Harrison's furrow. Expiration was pro-

* Submitted for publication March 27, 1913.

longed over the upper lobes of both lungs. Numerous moist râles were heard over the entire posterior aspect of the left lung and in the left lower axillary region. There was pronounced precordial bulging and diffuse heaving synchronous with the heart beat, the point of maximum intensity being in the sixth interspace slightly to the right of the left anterior axillary line. The cardiac impulse was marked and sudden and was preceded by a rough vibratory presystolic thrill. The right cardiac border was 5 cm. to the right of the mid-sternal line, and the left border 12 cm. to the left, at the third interspace. A presystolic rumble terminating in a sharp flapping first sound was heard at the apex and was followed by a short puffing murmur, heard over a wide area including the left axilla. The second sound at the pulmonic area was markedly accentuated and louder than that at the aortic area. The rhythm was regular. The abdomen showed marked epigastric pulsation. The blood, urine and feces showed no abnormalities.

Treatment.—The patient's condition remained unchanged for five days, during which period the pulse-rate averaged 120, the rhythm being always regular and the heart sounds continuing as noted at the first examination. It is not known whether digitalis had been administered before her admission, but on Jan. 22, 1912, the tincture

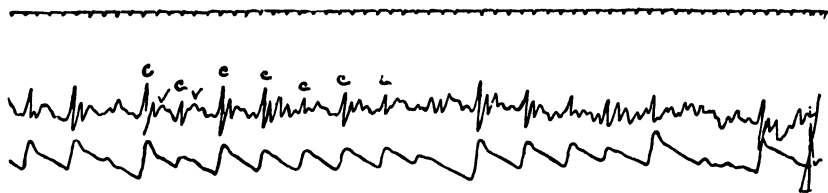


Fig. 1.—The upper line shows the time in 0.2 seconds. The second and third lines are curves of the jugular (venous) and radial pulses. Complete irregularity of the heart (auricular fibrillation) is present.

of digitalis was first prescribed in the hospital, the dose being 1 dram a day. After the administration of one-half dram, the patient was awakened by severe palpitation. The resident physician noted at this time that the apex rate had fallen to 80 and that the pulse was grossly irregular in rhythm and in force. The patient was not seen by the physician in charge until 2 drams of the tincture of

digitalis had been administered. The medication was then discontinued. It was noted at this time that the presystolic murmur and thrill had disappeared. In a polygraphic tracing taken January 24, the radial curve showed a *pulsus irregularis perpetuus*; and the venous, the ventricular type.

All subsequent tracings were of the same character, so that the conclusion was reached that auricular fibrillation was present. The slower rate of the ventricles continued until the patient was discharged from the hospital, Feb. 7, 1912. The change to a slower

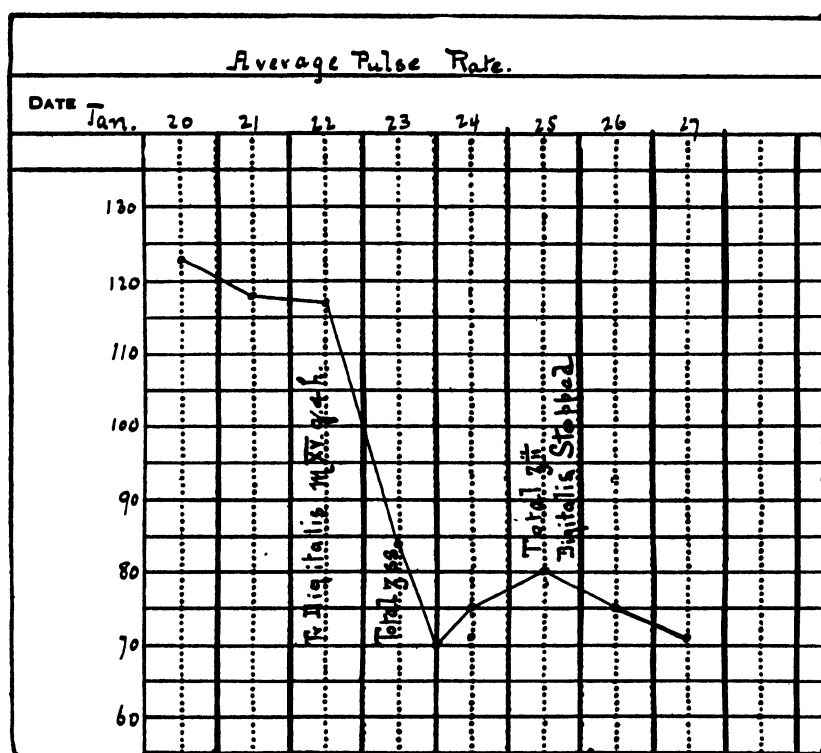


Fig. 2.—Chart showing average pulse-rate as affected by digitalis.

rate and an irregular rhythm coincided with rapid improvement in the general condition, and also in regard to dyspnea, cough and cyanosis. When the patient left the hospital, the circulation was restored to a condition of compensation.

Second Admission.—On her second admission, March 12, 1912, the patient was brought to the hospital in a condition of orthopnea. The temperature was 102.6 F.; the cardiac rhythm was completely irregular, the apex rate being 110; the respirations were 54 per minute. There was almost constant coughing with expectoration of much thin frothy fluid which did not contain blood. There was marked general subcutaneous edema. Auscultation of the chest disclosed the presence of numerous fine and coarse râles which could be heard over the entire posterior aspect; resonance was impaired at both bases. The abdomen was dome-shaped and flat on percussion in both flanks. The liver was pulsating and its lower border extended 7 to 8 cm. below the costal margin at the mid-clavicular line. The urine contained albumin and hyaline and granular casts. Next morning at 9:30, after having passed a fairly comfortable night, the patient was in no distress. One hour later she suddenly developed delirium cordis, with urgent dyspnea and general cyanosis. At this time Dr. H. G. Schleiter saw the patient with one of us in consultation, and it was decided to administer strophanthin intravenously, and to follow this by 1 dram of the tincture of digitalis daily by mouth. The injection was subsequently carried out by Dr. Schleiter, who has kindly furnished the following note and the accompanying tracing:

"March 13, 1912. At 11:20 a. m., the patient was in extreme distress, sitting up against pillows and panting for breath. The following conditions were noted: The lower extremities were extensively edematous. The right border of the heart was 2 inches to the right of the mid-line; the left border was 4 inches to the left in the third interspace; the apex, 5 inches to the left in the sixth interspace. The pulse-rate, taken at the apex, was 164 per minute and showed complete arrhythmia. Jugular tracings were not obtainable, but the radial pulse showed beats varying constantly in time and force, with no underlying dominant rhythm demonstrable; it does not appear reasonable to doubt that auricular fibrillation was present. Fluid was present at the bases of both pleural cavities. Many crepitant râles were heard. There was ascites; the liver pulsated and the edge was felt at the level of the umbilicus. The patient was given an intravenous injection of 0.5 milligrams stro-

phanthin. Within five minutes she appeared less ill, and in twenty minutes she was breathing quietly as if after a hypodermatic injection of morphin; the pulse, still showing fibrillation, had fallen to a rate of 116 per minute. A second injection of strophanthin (0.5 milligrams) was given at 3:30 p. m. It is of interest to note

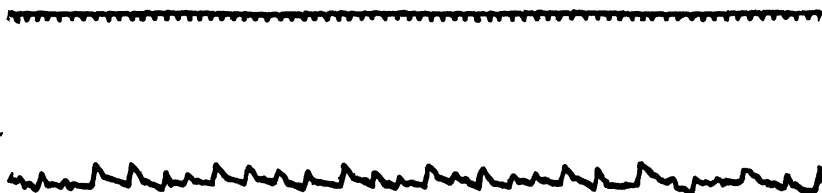


Fig. 3

Fig. 3.—A curve of the radial artery before the administration of strophanthin, 0.5 mgm., intravenously.

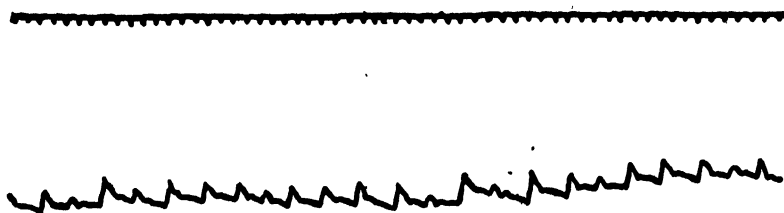


Fig. 4.

Fig. 4.—The same thirty minutes after the intravenous injection.

that after 7 drams of the tincture of digitalis had been administered, the pulse-rate had fallen to 84 per minute and the patient's condition had greatly improved. The heart outline had not been reduced, the characteristic irregularity was still present, and the liver border still remained at the level of the umbilicus, where it had been on admission to the hospital."

On the day following and for several days subsequent to the injection of strophanthin, considerable pain and swelling were present in the arm which had been chosen for its administration. Urgent subjective symptoms did not return. During the first three days



Fig. 5.—Microphotograph of the sino-auricular node. Slide 257. The pericardium is directed upward. The new-formed, thin-walled blood-vessels are seen in the subpericardial fat. The node has a triangular shape, base toward the pericardium.

after the beginning of the administration of digitalis, the rate of the heart occasionally reached 120 to 135, but subsequently it fell to normal and remained so after the withdrawal of the drug. Improvement was progressive. On March 24 the urine was free from

albumin and casts. The patient was discharged in April, 1912, with the circulation fairly well maintained.

Third Admission.—About five weeks later, on May 21, 1912, the patient was admitted for a third time. She said that she had been very comfortable since leaving the hospital, spending most of her

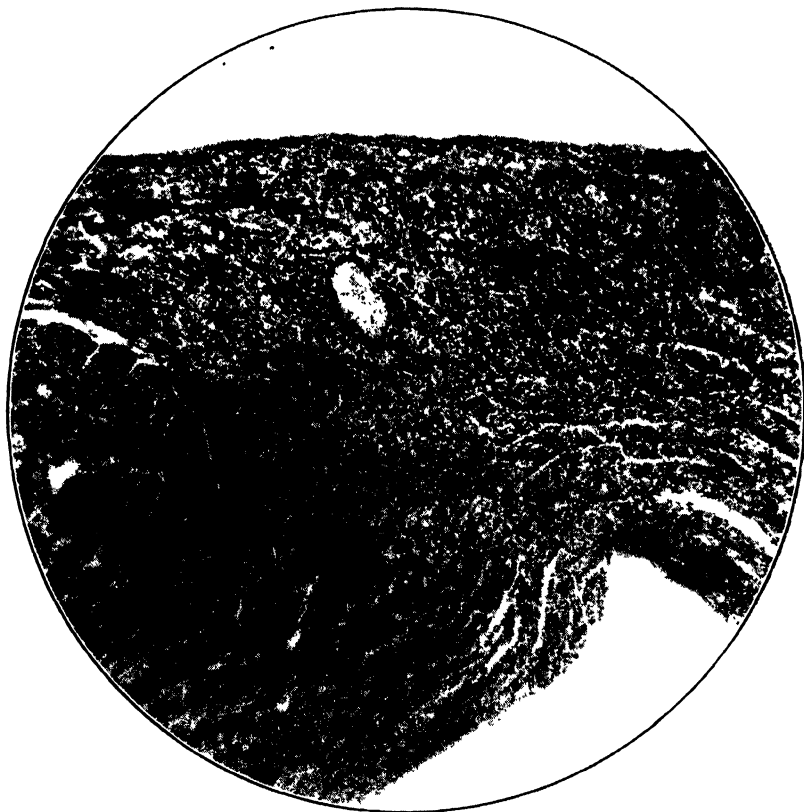


Fig. 6.—Microphotograph of the sino-auricular node. Slide 309. The node has an elongated form, its long axis parallel to the pericardium. A normal relation between muscle and connective tissue is seen.

time in her chair. She had taken no medicine. The evening before admission she began to be short of breath, the dyspnea soon becoming very urgent. On admission, the patient was found to be in a condition of marked decompensation. She had orthopnea,

cyanosis, general edema, anasarca and albuminuria. The temperature was normal; the respirations 40; the rate of the heart at the apex 160; the radial pulse was weak and intermittent, the average rate being about 100; the area of cardiac dulness was like that on the previous admission; the lower border of the liver was at the level of the umbilicus. There was constant coughing and expectoration of much white frothy fluid. The patient was given the tincture of digitalis. She had four hours sleep after two injections of one-fourth grain of morphin sulphate. She suffered less discomfort next morning, but in the evening had marked dyspnea, tossed about in bed and refused, at times, to answer questions. Strophanthin (1 milligram) was administered intravenously by the resident physician, but some of the solution escaped into the tissues so that it was not possible to determine the exact quantity which was absorbed. The patient gradually became more comfortable, but no striking change in her condition was noticed. May 23, 1912, the patient was very cyanosed and moved continually in bed. She was stuporous, but responded to questions. Her temperature was normal; the heart rate at the apex was 120, and the radial pulse 80. After a vein had been exposed by dissection, strophanthin (1 milligram) was injected into it, but without apparent result on the heart rate or mass movement of blood. Two days later, May 25, 1912, the temperature suddenly rose to 105.4 F., without a premonitory chill. The patient was very restless. There was marked icterus. The leukocytes numbered 15,000, and in the differential count there were 95.6 per cent. polynuclear cells. Twelve ounces of blood were withdrawn by venesection. During May 26, 1912, the temperature again reached 105.4 F. The patient was very restless and cyanotic. On the next day there was progressive failure of the circulation. The cardiac impulse was tapping in character, and the rhythm was grossly irregular. Pulmonary edema set in. The temperature ranged between 101 and 106 F. Death occurred May 28, at 2:30 a. m. Blood-culture showed the presence of an unidentified streptococcus.

An autopsy was performed several hours later by Dr. Oskar Klotz, to whom we are indebted for the report of the post mortem examination.

NECROPSY.

The body was that of a slightly built woman of about middle age. The nutrition was fair. The pupils were both dilated, the left being larger than the right. The breasts were small and atrophied. There was some excoriation of the skin over the sacrum. The external orifices were without change. The skin tissue everywhere showed a marked yellow pigmentation, as did also the sclera.

Neck Organs.—The trachea was a little congested but did not contain excessive exudate. The lymph-nodes at the bifurcation of the trachea showed a single small calcareous nodule the size of a mustard seed. The esophagus showed no change. The thyroid was not enlarged but was rather meaty looking. The tissue of the neck below the thyroid gland appeared edematous, and in it were several dark red lymph-nodes.

Thorax.—There were some old adhesions along the posterior border of each lung. The apex on each side was free. There was no excess fluid in either pleural cavity. The pericardium contained about 75 c.c. of a clear yellow fluid.

Left Lung.—The organ crepitated throughout. The tissues appeared a little heavy, but there was no evidence of edema. There was only a slight marbling of black pigment. On section the lung substance was a little congested. The arteries of the lung showed some irregular thickening of their walls. The hilus nodes were not enlarged. The bronchi appeared quite clear.

Right Lung.—The organ was very similar to that on the left side. There was some slight congestion but no edema. In one of the main pulmonary arteries there was a flat area showing adherent grayish red clot. This clot did not obstruct the artery. The hilus nodes were without change.

Heart.—See below.

Aorta.—The aorta showed some diffuse thickening of the intima in the arch and in the descending thoracic portion. The carotid arteries showed some irregular nodular areas near the bifurcation.

Abdomen.—On opening the abdomen the partially distended small intestine protruded. The great omentum showed an adhesion to the right of the broad ligament. The spleen was connected by old adhesions to the diaphragm. There were some old bands of fibrous tissue between the left lobe of the liver and the diaphragm. The left ovary had some adhesions to the sigmoid. There was no excess fluid in the peritoneal cavity.

Alimentary Canal.—The stomach and intestines showed nothing unusual. There was some congestion of the lower portion of the small and large intestines. The mesenteric lymph-nodes were not enlarged.

Liver.—The organ was rather swollen and was flabby. The outer surface of the left lobe showed some old adhesions. The organ was quite pale in color. The gall-bladder contained thick brown bile, which contained many pigmented granules. The liver on section was bright yellow, in which some dark red spots and streaks could be distinguished. The lobules were not distinctly marked. There was no evidence of fibrosis. The yellow areas were not definitely depressed but some resembled areas of necrosis.

Spleen.—The spleen was tense and firm. On section the organ appeared dark and firm in certain areas, while in others the tissue was soft and could be readily scraped with a knife. The softer areas appeared more particularly in the center of the organ. The Malpighian bodies were indistinct.

Kidneys.—Both organs were very much alike. In each instance the capsule was quite thin and not adherent. The cortex was gray and somewhat swollen; it was poorly defined from the medulla. The pelvis and ureters were normal.

Adrenals.—Both adrenals were quite large. The cortex was red and showed no evidence of fat. The medulla was not enlarged and was of a gray color.

Bladder.—The organ was of good size. The walls were thin and pale.

Genitalia.—The tubes were both normal. The ovary in the left side contained a cyst 2.5 cm. in length. The uterus was normal in size and appearance. The cervix showed the presence of two old tears. In the vagina there was some excoriation of the epithelium on the posterior wall just below the cervix.

MICROSCOPICAL REPORT.

Lymph-Nodes.—The lymph sinuses throughout were much dilated, as were also the blood-vessels. The sinuses were filled with lymphocytes, a few polymorphonuclear leukocytes and occasional endothelial cells. There was much carbon pigment.

Lung.—The alveoli were of usual size. Their walls were thin and there was no evidence of any inflammatory exudate into the alveoli.

Heart.—Sections of the heart muscle showed a rather poorly staining tissue in which the muscle fibers did not stain evenly. The muscle fibers appeared rather narrow. The striations of the muscle fibers were indistinct and these were seen in broken fragments in the neighborhood of some of the blood-vessels. About these blood-vessels was an excess amount of connective tissue. In other places the capillaries lying between the muscle bundles were distended with blood with occasional extravasation of red blood-cells into the surrounding tissue. Here and there a number of polymorphonuclear leukocytes were seen in the interstitial tissue. Portions of the heart muscle cut on the freezing microtome showed an abundance of fat deposited within the muscle cells. This fatty degeneration was irregularly distributed through the tissue; in places it was very marked. Islands of connective tissue were also evident in the vicinity of the blood-vessels.

Thyroid.—Sections of the thyroid gland showed tissue with many medium sized alveoli containing colloid material. The alveolar walls were quite thin and the epithelium somewhat flattened.

Kidney.—In the cortex of the organ the glomeruli were of good size, and they commonly showed fairly wide capsular spaces. The tubules of the cortex were large, many of them had wide lumina and contained some debris. The cells lining the tubules were of irregular size and stained poorly.

Liver.—The structure of the organ was much altered. The central zones of the lobules were almost entirely destroyed, there being no liver columns in these regions. The sinuses were much dilated and there appeared to be an extravasation of red blood cells into the surrounding tissues. Each lobule showed only a rim of liver cells in the periphery.

Spleen.—The organ was intensely congested so that the normal markings were almost obliterated. The Malpighian bodies were small.

BACTERIOLOGICAL REPORT.

Blood taken a day before death contained streptococci. The biologic character of this organism was not identified.

Pathologic and Bacteriologic Diagnosis.—(Rheumatism); chronic sclerotic mitral endocarditis; stenosis of mitral valve; chronic interstitial myocarditis; chronic sclerotic aortic endocarditis (slight); dilatation of heart (left ventricle and right auricle); hypertrophy of heart (left ventricle); milk spots of heart; fatty degeneration of heart; acute interstitial myocarditis; hydropericardium; thrombosis of pulmonary artery; sclerosis of pulmonary artery; old bilateral pleural adhesions; obsolete tuberculosis of peribronchial glands; chronic congestion of lung; peritoneal adhesions; chronic perisplenitis; cardiac spleen with acute splenitis; chronic perihepatitis; nutmeg liver; bile sand; central necrosis of liver; hemorrhage into liver; cloudy swelling of kidney; enlarged adrenals; cyst of ovary; chronic perisalpingitis; old laceration of cervix; decubitus ulcer of sacrum and vagina; septicemia (streptococcus).

TECHNIC OF EXAMINATION OF HEART.

Heart.—The heart was fixed in Zenker's solution. The area containing the sino-auricular node was washed and hardened in alcohol. The rest of the heart was taken out of Zenker's solution, washed in running water and then sent for examination in a 1 per cent. formaldehyd solution. It was then washed and preserved in alcohol. The length of the heart in the fixed state was 12 cm. from the auriculo-ventricular groove to the apex on the anterior surface, and 9 cm. on the posterior surface. There was a moderate amount of subpericardial fat along the right border of the heart; here there was a milk spot measuring 4 by 2.5 cm. A similar one measuring 1.5 by 1.5 cm. was found near the apex on the anterior surface of the right ventricle. The cavity of the right auricle was dilated but not hypertrophied. The endocardium was white and somewhat yellowish. A small Chiari net guarded the opening of the coronary sinus. The tricuspid valve admitted three fingers; the borders of the flaps were thickened. The right ventricle was neither dilated nor hypertrophied. The pulmonary valve was normal. The cavity of the left auricle was much dilated. The endocardium was thick and white. There were no vegetations. The mitral valve admitted two fingers. The edges of the flaps were much thickened and were rolled in on the auricular surface. The cavity of the left ventricle was slightly dilated but not hypertrophied. The wall measured 15 mm. at the base, 17 mm. at the level of the papillary muscles and 8 mm. at the apex. The outflow tract of the ventricle was more dilated than the left half of the cavity, and the endocardium lining it was white. The chordae tendineae were shorter and slightly thicker than normal. There were two small false posterior moderator bands. The corpora Arantii of the cusps of the aortic valve were thickened. The cusps themselves were adherent to each other at their points of intersection; otherwise they were normal. The coronary arteries were normal. The heart presented no congenital anomalies.

Sino-Auricular Node.—The area at the cavo-auricular junction containing the sino-auricular node was hardened, embedded, cut and stained in the manner employed and described elsewhere.² The sections were cut at a thickness of

12 microns at right angles to the long axis, and every fifth section was mounted. On account of the fixation in Zenker's solution, bichlorid precipitation occurred but was removed successfully by immersing the sections in Lugol's solution for one week before staining. The number of sections cut was 2,235, so that the length of the node after embedding was 26.82 mm.³ The relation of the upper extremity of the node to the cavo-auricular junction could not be ascertained because the tissues had been excised from the heart before it was sent for examination, and the normal morphologic landmarks were consequently destroyed. The shape and dimensions of the transverse section of the node varied at different levels. In its upper extremity it was circular and its diameter was 1 mm. It lay directly under the pericardium, the wall of the auricle measuring 3.5 mm. There was a small hemorrhage close to the node. In the subpericardial fatty and connective tissue and also sometimes encroaching on the substance of the node itself, but confined almost wholly to the nodal area, there were many thin-walled blood-vessels, probably inflammatory or degenerative in origin. All of these, as well as the other vessels seen in this series, showed very marked congestion. A short distance below its upper extremity, the node lay 2 mm. deep from the pericardium and precisely at the junction of the taenia terminalis and the atrium. It measured 4 by 0.5 mm., the long axis being parallel to the pericardium. The shape was roughly triangular, except that the angles were much rounded off. The structure of the node, aside from the abnormalities noted, was normal, both in the total quantity of muscle present and in the relation of this amount to that of connective tissue. An unusual arm projected outward from its left border toward the pericardium, continued parallel with it a short distance and was finally lost in the muscular structure of the auricle. At a level still lower, the node measured 6 by 1 mm. and lay only 0.5 mm. from the pericardium. The largest proportions the node attained were 6.5 by 1.5 mm., 13.74 mm. from the upper extremity. From this point onward, the dimensions of the node became rapidly reduced and the structure terminated in a tail, the larger part of which was formed of connective tissue.

The relation of muscle to nerves and ganglia was unusually close. At all levels there were collections of ganglion cells in the space between the pericardium and the muscle layer. Nerve bundles, both small and relatively large, were found in the same situations and also within the structure of the node itself. About the nerve ganglia and nerve fibers the new-formed, thin-walled blood-vessels, which have been described, were found in rather large numbers. The artery, which is so often found in relation to the node, had at a low level a distinct reduction in the diameter of the lumen, due to an endarteritic process. This vessel showed no other point of interest except collections of smooth muscle fibers arranged in bundles and irregularly distributed about the vessel as an external longitudinal layer, similar to that which has been observed to lie about the central venous sinuses of the adrenal glands.

The Conduction System.—The block containing this system was excised in the usual manner. It was cut in sections 12 microns thick in a plane at right angles to the long axis of the heart. Every fifth section was mounted. In the inter-auricular septum both muscle tissue and fat showed the presence of many leuko-

3. The length of the node in another heart weighing 899 gm., already reported (Heart, 1912, iv, 24) measured 21.55 mm. Koch (*Med. Klin.*, 1911, No. 12) gives the length as about 3 cm., but cites no specific examples.

cytes and lymphocytes in groups and also lying between the strands of muscle fibers. The auriculonodal junction was of sufficient size but was inflamed. The auriculoventricular node was of normal size, large rather than small, showing the characteristic interlacing of the fibers, but poorer in nuclei than is usual. There was a fairly well marked inflammatory process of the nature described and also moderately large collections of inflammatory cells within the substance of the node. A similar inflammatory lesion was also found near the beginning of the main stem. It lay just outside the bundle and extended a short distance along the sheath. It did not compress the bundle. The main stem was large, well-developed, normal in appearance and intact from auricles to ventricles. Just before the division of the main stem into the ventricular branches, there was another area showing an infiltration with leukocytes and lymphocytes. From the point of division onward, the branches, more especially the right, showed only slight inflammatory lesions. Both branches were normal in size, the right a little larger than the left at the beginning.

Résumé.—The sino-auricular node showed normal relations between the amounts of connective tissue and muscle; there was no sclerosis, but an inflammatory lesion in slight part within the substance, but for the most part without, though in the neighborhood of the node. The auriculoventricular node was inflamed to a far greater extent than the sinus node and contained abscess-like bodies. Similar smaller collections were found in the main stem. Congestion was a marked feature of the lesion throughout. There was no break in continuity of the conduction system.

DISCUSSION AND SUMMARY.

A case is described in which, while the patient was under observation, a sequential rhythm changed to complete irregularity of the cardiac mechanism, due to auricular fibrillation. It is extremely doubtful whether the one-half drachm of digitalis which the patient received could have been responsible for the altered rhythm, even if she had been given the drug before admission to the hospital, five days before. Neither can the fall in ventricular rate from 120 to 80, occurring with the onset of fibrillation, be ascribed to an administration of digitalis extending over only half a day. The fall in rate occurring with the onset of auricular fibrillation is difficult to explain, but it occurs in other conditions of which we have only slight knowledge. The action of strophanthin administered intravenously was studied and showed a strikingly beneficial effect on the circulation and the subjective symptoms of the patient. The post mortem examination showed a lesion of the sino-auricular node, but it cannot be definitely asserted that a relation exists between the lesion described and the clinical irregularity that was studied.

THE OXYGEN CONTENT OF THE BLOOD IN RABBITS INFECTED WITH PNEUMOCOCCUS.*

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New York.)

It has been shown by experiments *in vitro*¹ that the growth of the pneumococcus on blood decreases the capacity of the blood for combining with oxygen. This effect is seen when the pneumococcus is planted in mixtures of unlaked red corpuscles, but it is more marked in solutions of hemoglobin, and the lowering of the oxygen-combining power is apparently due to the formation of methemoglobin. Even when spectroscopic examination fails to show the absorption bands of methemoglobin, the oxygen-combining power of the hemoglobin is decreased, and spectrophotometric measurements make it certain that methemoglobin is present. The determination of the power of the blood for combining with oxygen is thus a very delicate test for changes taking place in it.

As a result of these experiments *in vitro* it seemed of importance to determine whether similar effects on the blood took place in animals with a pneumococcus septicemia. For this purpose rabbits were used, and the animals were injected with large doses of pneumococcus so as to induce rapidly a profound bacteremia. They were inoculated in the ear vein with the organisms centrifuged out from 200 to 500 cubic centimeters of an eighteen hour bouillon culture. Shortly after inoculation the organisms could be found in smears of the blood, and the number of organisms increased until at death enormous numbers were present. Clinically the most striking symptom was the abnormal respiration. Most of the animals showed a polypnea, but several had a slow, deep respiration, not unlike the typical breathing of diabetic acidosis. As the infection

* Received for publication, March 27, 1913.

¹ Butterfield, E. E., and Peabody, F. W., *Jour. Exper. Med.*, 1913, xvii, 587.

became more marked, a cyanosis of the mucous membranes and conjunctivæ developed and the blood in the ear veins became much darker. Death usually occurred in from four to six hours.

The blood after death varied from a dark reddish brown to a chocolate color. When shaken with air it changed color rather slowly and often failed to become bright red. The coagulation time was increased. Sometimes the blood remained fluid for many hours. The serum was yellowish green and turbid, much as described by Longcope² after the growth *in vitro* of pneumococcus in the sera of pneumonia patients. The reaction of the serum to litmus paper was variable. Sometimes it decolorized litmus paper, sometimes it gave a slightly alkaline or an amphoteric reaction, and occasionally it was acid to litmus a short time after death. In general it was, however, much less alkaline to litmus than is normal rabbit serum. The carbon dioxide content of the blood was determined in several animals, and a marked falling was observed as the infection advanced. This is, of course, exactly what occurs in other types of acidosis. Spectroscopic examination of the laked red cells usually showed the absorption bands of oxyhemoglobin or hemoglobin. Only very rarely were the bands of methemoglobin present, although they occasionally appeared after the blood had been kept for some hours *in vitro*.

Observations were made by the Barcroft and Haldane³ method on the oxygen content of blood from the carotid artery at various times after the animal was inoculated, and on the power of the blood for combining with oxygen at the same time and also immediately after death. The combining power was tested by laking the blood and shaking it for five minutes in air in order to saturate it with oxygen. A source of error exists in these determinations in that the pneumococcus, an organism which uses up oxygen rather rapidly, was present in the blood. There was undoubtedly some loss of oxygen during the analysis of the specimen, but all specimens were analyzed as nearly as possible at the same rate and with the same precautions, and control observations on the rate of destruction of oxygen by the organism in specimens of blood taken

² Longcope, W. T., *Jour. Exper. Med.*, 1905, vii, 626.

³ Barcroft, J., and Haldane, J. S., *Jour. Physiol.*, 1902, xxviii, 232

after death made it clear that the error was not large enough to affect the general meaning of the results. This source of error was, moreover, finally controlled and in great part eliminated in experiment 8. Blood was taken before inoculation and immediately after death. Both specimens were laked and centrifuged rapidly, so that smears from the blood taken after death contained no pneumococci. This sterile solution of hemoglobin showed a decrease in the oxygen-combining power of over 17 per cent.,—a change exactly similar to that found in the other experiments. It is thus evident that the low oxygen values are not dependent on the presence of organisms in the blood during the analysis, but on a change in the hemoglobin molecule (table I).

The results may be briefly summarized as follows:—The first specimen of blood was taken as a control before inoculation or before the animal showed marked symptoms. It gives the normal oxygen content of the arterial blood for the individual animal, and the content of the blood when saturated with oxygen. Subsequent specimens show a diminution both in the oxygen content and in the oxygen-combining power of the blood. The degree of this diminution is more or less parallel to the severity of the infection, and the fall is progressive until death occurs. At the time when death took place, the oxygen-combining power of the blood was usually between 60 and 75 per cent. of its original value. In some of the experiments the oxygen content fell in approximately the same ratio as the oxygen-combining power. In these, then, the degree of saturation with oxygen remained about normal. In other instances (rabbits 1 and 2), however, the oxygen content fell more rapidly than the combining power, and the arterial blood was much less completely saturated than normally. This low degree of saturation might depend in part on the fact that oxygen is directly used up by the pneumococcus, for it is possible that if the venous blood reached the lungs with an abnormally low oxygen percentage, the time of the passage of the lung capillaries would not be long enough to allow the blood to become completely saturated. On the other hand, as there is a gradual shift in reaction of the serum, and as the hemoglobin molecule is ultimately profoundly changed by the

TABLE I.

The Oxygen Content of the Arterial Blood, and the Oxygen-Combining Capacity of the Blood of Rabbits Infected with Pneumococcus.

Time of inoculation.	Time at which blood was taken.	Per cent. of oxygen in arterial blood.	Per cent. of oxygen in blood when completely saturated.	Remarks.
Rabbit 1. Oct. 22, 1912. Inoculated at 10.45 A.M.	10.30 A.M.	10.81		Control specimen before inoculation.
	12.55 P.M.	1.47		Animal dying. Arterial blood reddish brown.
	1.00 P.M.		7.08	Blood from heart. Heart still beating when chest was opened. After standing 24 hrs. this blood, in strong solution, shows absorption bands of methemoglobin. It decolorizes litmus paper, but does not turn it red.
Rabbit 2. Oct. 24, 1912. Inoculated at 10.25 A.M.	12.30 P.M.	7.12	8.88	No observation made while animal was still normal.
	1.05 P.M.	2.82	6.78	Death at 1.25 P.M. No methemoglobin absorption bands.
Rabbit 3. Oct. 29, 1912. Inoculated at 12.45 P.M.	2.30 P.M.	11.40	12.51	
	4.30 P.M.	10.01	11.20	
	5.00 P.M.		9.79	Blood taken after death. Heart still beating.
Rabbit 4. Nov. 1, 1912. Inoculated at 9.15 A.M.	11.25 A.M.	14.34	18.59	
	12.10 P.M.	13.22	16.37	Dyspnea.
	12.45 P.M.	11.14	12.95	
	1.40 P.M.		9.89	Blood taken from heart at death. Heart still beating.
Rabbit 5. Nov. 7, 1912. Inoculated at 9.20 A.M.	11.40 A.M.	12.11	14.80	
	1.00 P.M.	8.02	9.25	
	1.15 P.M.		9.05	Blood taken 5 min. after death. No absorption bands of methemoglobin.
Rabbit 6. Oct. 12, 1912. Inoculated at 9.00 A.M.	3.25 P.M.		12.21	
	4.25 P.M.		10.12	Oxygen content of venous blood, 2.06 per cent. at 4.00 P.M.
	5.20 P.M.		8.81	
	5.40 P.M.		7.71	Rabbit dying.
Rabbit 7. Oct. 8, 1912. Inoculated at 9.00 P.M.	12.30 P.M.		10.28	Control observation before inoculation.
	7.15 P.M.		7.00	Blood taken shortly after death.

growth of the pneumococcus, it is not unlikely that there is a slowing of the reaction between the hemoglobin and oxygen. It is noticeable that when one shakes specimens of infected blood in the air, they appear to become red unusually slowly, but it has not been possible to make any direct observations on the rate with which these bloods take up oxygen.

In pneumococcus septicemia in rabbits, then, the blood is changed so that it takes up less oxygen than normally. This change only begins to appear when the infection is advanced. It is progressive and reaches its maximum at death. Associated with this fall in the oxygen-combining power is a progressive fall in the oxygen content of the arterial blood. The fact that the latter is often relatively greater than the former suggests a slowing or other interference in the reaction between hemoglobin and oxygen. Occasionally specimens of infected blood show the absorption bands of methemoglobin.

The changes in the infected rabbit are exactly analogous to the changes occurring when the pneumococcus grows in blood *in vitro*. In solutions of hemoglobin, practically all the hemoglobin may be converted into methemoglobin. In mixtures of unlaked red blood cells the action is less intense and after twenty-four hours' growth methemoglobin cannot usually be found spectroscopically. There is, however, a profound fall in the oxygen-combining power of the blood, and spectrophotometric measurements show that the change to methemoglobin is taking place. In infected rabbits the change in the blood rarely goes on so far that methemoglobin is distinguishable spectroscopically, for the animals die before this stage is reached. The fall in the oxygen-combining power of blood, however, shows that the hemoglobin is being affected, and this, together with the occasional finding of methemoglobin in the blood from animals, makes it certain that in animals the growth of the pneumococcus produces the same effect on the hemoglobin molecule as it does *in vitro*. It is impossible to decide definitely whether acid production plays a part in the alteration of the hemoglobin, but experiments *in vitro* are contrary to this supposition.

CONCLUSIONS.

In rabbits with a severe pneumococcus bacteremia, the oxygen-combining power of the blood falls progressively up to the time of death. Coincident with this there is an even more marked fall in the oxygen content of the arterial blood.

The changes in the blood in infected animals are analogous to those seen when the pneumococcus is grown on blood *in vitro*. They are due to the conversion of hemoglobin into methemoglobin.

THE OXYGEN CONTENT OF THE BLOOD IN LOBAR PNEUMONIA.*

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New York.)

For two reasons it seems possible that a study of the oxygen content of the blood in pneumonia might yield results of interest. In the first place, the disease affects primarily the lungs, so that a disturbance of the respiratory exchange of gases might take place. In the second place, the growth of the pneumococcus produces a marked change in the hemoglobin molecule. Butterfield and Peabody¹ have shown that, as a result of its growth on blood *in vitro*, the hemoglobin may be converted into methemoglobin. As yet, however, practically no direct determinations of the oxygen content of the blood in pneumonia have been made. The following study was therefore undertaken to obtain some evidence as to the extent and manner in which the disease affects the oxygen of the blood, and the relation of any changes in the oxygen to the symptoms and course of the disease. Analyses of arterial blood were, of course, greatly to be desired, but in a study on patients it seemed safer to use only venous blood. Since this work was finished, however, Hürter² has shown that it is both safe and comparatively easy to take blood from the radial artery. While analyses of peripheral venous blood are not wholly satisfactory, there are certain facts of interest which are brought out by them, and which may serve as a basis for further study.

The method used was that of Barcroft and Haldane,³ as modified by Brodie.⁴ This has been briefly described, together with the

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¹ Butterfield, E. E., and Peabody, F. W., *Jour. Exper. Med.*, 1913, xvii, 587.

² Hürter, *Deutsch. Arch. f. klin. Med.*, 1912, cviii, 1.

³ Barcroft, J., and Haldane, J. S., *Jour. Physiol.*, 1902, xxviii, 232.

⁴ Brodie, T. G., *Jour. Physiol.*, 1909-10, xxxix, 391.

technique of taking the samples of blood, in a previous paper⁵ in which the results of the analyses of the same bloods for carbon dioxide were discussed. It is perhaps well to repeat here that the blood was taken without obstructing the flow in the veins. Nearly all observations were made in duplicate, and few analyses had to be discarded on account of the results failing to agree.

Control observations were made on normal individuals and on patients during convalescence from pneumonia. Twenty-one specimens of blood from seven convalescents and five normal adults showed an oxygen content varying from 9.23 to 15.02 per cent. Of these only three, all from convalescents, were below 10 per cent., and only one, from a normal individual, was above 14 per cent. It is thus important to realize that with subjects at rest and in what may be considered normal conditions, the oxygen content of the peripheral venous blood may vary within rather wide limits. Buckmaster and Gardner⁶ report a series of eleven observations made by a different method on their own venous blood, and found the oxygen content to range between 2.63 and 10.8 cubic centimeters per 100 cubic centimeters of blood. In the present study figures as low as some reported by these authors were rarely met with, and then only under pathological conditions.

The material for study consisted of twenty-five cases of lobar pneumonia. In many cases repeated observations were made during the course of the disease and also in convalescence. The total number of determinations was eighty. The results obtained may be conveniently considered in three groups.

The first group of observations was made on cases in which the disease was running an uncomplicated course and in which no abnormality of the oxygen content of the blood was found. This series consists of forty analyses of blood from fifteen cases. In seven cases only a single specimen of blood was taken in the febrile period, but in others as many as six or eight observations were made during the disease and in convalescence. Twenty-seven of the samples were taken during fever and thirteen at a time when

⁵ Peabody, F. W., *Jour. Exper. Med.*, 1912, xvi, 701.

⁶ Buckmaster, G. A., and Gardner, J. A., *Proc. Roy. Soc.*, 1912, lxxxv, Ser. B, *Biological Sciences*, 56.

the temperature had become normal. The earliest were taken on the third, the latest on the eighteenth day of the disease. In all of the forty observations the oxygen content was between 9.03 and 14.52 per cent., while only four specimens gave more than 14 per cent., and only six gave below 10 per cent. Several cases had a rather higher oxygen content during the febrile period than during the subsequent afebrile period.

This group of cases, which constitutes by far the greater number of uncomplicated instances of pneumonia, shows, then, an oxygen content of the peripheral venous blood which is well within the normal limits. The differences found during the various stages of the disease and the differences between the febrile and convalescent periods do not exceed those found in health. The extent of lung involvement appears to have little influence on the oxygen. In one case the temperature was 103° F. and the rate of respiration thirty-two on the day before the crisis, and the oxygen content was 14.52 per cent. On the following morning the temperature had dropped to 98° F. and the respiration to twenty-four, but the oxygen was 13.55 per cent., in spite of there being no noticeable change in the physical signs in the chest. There is no definite relation between either temperature or respiration and oxygen content, except the rather obvious fact that during the acute stage of the disease the normal oxygen level is associated with a more or less increased respiratory rate. By this increase in the rate of respiration, and perhaps by a more rapid circulation, the organism is thus able to compensate fully for the decrease of respiratory surface caused by the pulmonary exudate. Such compensation is easy, for the lungs have a comparatively large factor of safety. Loewy and von Schrötter⁷ state that if one lung were completely shut off by the occlusion of its main bronchus, the venous blood would contain 8 per cent. of oxygen instead of the normal 14 per cent., even if there were no compensation on the part of the respiratory or circulatory systems.

The second group of cases consists of only two patients. Like those in group I, both ran an uneventful course ending in recovery.

⁷ Loewy, A., and von Schrötter, H., *Ztschr. f. exper. Path. u. Therap.*, 1905, i, 197.

Both, however, had a diminution in the oxygen content of the blood during the height of the disease. In the first case this diminution was only found once, on the fourth day of illness. The oxygen was then 5.67 per cent., but two days later it was normal and it remained so afterwards. In the second case the low oxygen was found at three separate times during two days. The lowest observation was 2.77 per cent. Before and after this period the oxygen was normal. Both cases were rather more cyanotic than was usual. Blood cultures were negative in both. In both cases the low oxygen figures were associated with values for carbon dioxide which were much higher than normally. The combination of low oxygen and high carbon dioxide suggests some pulmonary or circulatory interference with the normal gaseous exchange. In the second case, however, several observations showed that the blood lacked the power of taking up oxygen normally, even when shaken vigorously in the air. It is thus not improbable that there had been some change in the hemoglobin, and the case is somewhat similar to case 8 which will be discussed in more detail below.

The third group consists of those cases which terminated fatally. Death in pneumonia may result in a variety of ways. It often comes suddenly as the result of cardiac or of respiratory failure. It may be the result of a complication. Not infrequently, however, the clinical history is somewhat as follows: Towards the end of the first week of the disease the patient, who has been doing well, begins to show serious symptoms. The pulse becomes rapid, weak, and perhaps irregular. Respiration is shallow, labored, and rapid. Cyanosis becomes marked and toxemia deepens. After some hours coarse, moist rales begin to appear in the chest. A general pulmonary edema, associated with coarse tracheal rales, develops, the patient becomes increasingly weak and toxic, and dies in twelve to twenty-four hours. This more or less gradual type of death is the one which is most frequently met with, and was present in the majority of the fatal cases in this series. Dochez⁸ has analyzed a number of these typical, slowly progressive cases, with a view to finding the actual cause of death. He concludes that in general the patient can withstand the original infection, and that

⁸ Dochez, A. R., *Jour. Exper. Med.*, 1912, xvi, 680.

death is due to a spreading of the infection and to the failure of the patient to resist the secondary insult. The spread of the infection may be in either or both of two ways. There may be a further involvement of the lungs, or there may be a spread to the blood and the development of an intense bacteremia. The degree to which the infection may spread in the lungs or in the blood before death ensues depends, of course, on the resistance of the patient.

Eleven fatal cases are reported here. Ten of these were of the slowly progressive type just described. One case (No. 11) died very suddenly, apparently of respiratory failure. There was no pulmonary edema and the heart sounds were audible for three minutes after respiration stopped. Blood cultures were made in ten cases. In nine of these they were positive, and bacterial counts by Dochez (table I) show that there was often a bacteremia of progressively increasing intensity. The number of organisms was frequently over 1,000 per cubic centimeter of blood at the time of death. In one case (No. 8) blood cultures were negative, even on the day of death, but the physical signs and autopsy showed a rapidly spreading pulmonary lesion.

Observations on the oxygen content of the blood during life were made in ten of the eleven fatal cases. In the one case that died suddenly (No. 11), the oxygen content of the blood was normal two hours before death. In one case (No. 10) the last specimen of blood was taken twelve hours before death and showed a normal oxygen content. In the other eight cases observations were made one or more times during the final stage of the disease and in every case the oxygen content was below normal. Moreover, these cases show (table I) that during the terminal stage the fall of the oxygen content of the blood is, roughly, a progressive one. Thus samples of blood taken from different cases ten, five and one half, and three hours before death contained 6.89, 8.40, and 8.12 per cent. of oxygen, respectively. Others taken two hours, one hour, and thirty minutes before death contained 4.68, 2.30, and 2.07 per cent. of oxygen. A specimen taken five minutes after death contained no oxygen. In several cases (Nos. 3, 4, 5, and 8) the oxygen content of the blood had previously been found to be normal, and the fall in the oxygen content accompanied the develop-

TABLE I.

The Oxygen Content and the Oxygen-Combining Power of the Blood in the Fatal Cases of Pneumonia.

No. of case.	Date (1912).	Per cent. of oxygen content of blood.	Per cent. of oxygen-combining power of blood.	Blood culture. ⁹	Remarks.
1 T. W. 525	Mar. 29, 10 A.M. 10 P.M. Mar. 30	7.80 8.40	 16.35 7.87	4,500 colonies per 1 c.c. of blood	Marked cyanosis. Many coarse rales. Blood taken 10 min. after death.
2 H. L. 453	Jan. 31	2.30			Cyanosis. Edema of lungs. Blood taken 1 hr. before death.
3 M. M. 533	Mar. 29 Apr. 2 Apr. 3	9.03 2.07	16.02 13.23	Positive on Mar. 28. 15,000 colonies per 1 c.c. of blood	Cyanosis. Many coarse rales. Blood taken 30 min. before death. Hemoglobin, 96 per cent.
4 T. C. 423	Jan. 15 Jan. 16	10.97 4.68		Positive on Jan. 14	Many coarse rales. Cyanosis not marked. Blood taken 2 hrs. before death.
5 J. McL. 438	Jan. 25 Jan. 26	10.41 6.89		100 colonies per 1 loop of blood	Marked cyanosis and edema. Blood taken 10 hrs. before death.
6 J. R. 547	Apr. 9 Apr. 11 Apr. 13 Apr. 15 Apr. 16	8.27 6.86 3.94 8.47	 16.29 16.71 13.95	Negative 4 colonies per 1 c.c. of blood 21 colonies per 1 c.c. of blood	Slightly cyanotic. Hemoglobin, 88 per cent. Cyanosis marked. Hemoglobin, 88 per cent. Cyanosis less marked. Died suddenly. Blood taken 10 min. after death. Serum reacts alkaline to litmus paper.
7 N. A. 544	Apr. 6 Apr. 8 Apr. 12	3.61 4.44	18.59 17.50 15.09	Negative on Apr. 5 Negative on Apr. 9 2,500 colonies per 1 c.c. of blood 1 hr. after death	Cyanosis very marked. Pulse weak. Many rales in chest. Blood taken 2 min. after death.

⁹ The blood cultures were made by Dr. Dochez.

TABLE I.—*Continued.*

No. of case.	Date (1912).	Per cent. of oxygen content of blood.	Per cent. of oxygen- combining power of blood.	Blood culture.	Remarks.
8 J. D. 460	Feb. 2	10.81	15.07	Negative	Coarse bubbling rales throughout chest.
	Feb. 3	11.98			
	Feb. 4, 4.30 A.M.	6.84			
	9.30 A.M.	9.07			
	2.30 P.M.	9.71			
	9.45 P.M.	12.09			
	Feb. 5 10.00 A.M.	8.12			
	1.00 P.M.	0.00			
	9 I. J. 535	Mar. 4		16.38	125 colonies per 1 c.c. of blood
10 W. H. 472	Feb. 12	7.78	16.84	Negative	Cyanotic and exhausted after a long walk to the hospital.
	Feb. 13	12.64		Positive Positive	
	Feb. 14	11.93			
	Feb. 15	12.71			
11 W. H. 480	Feb. 21	13.28	19.35	150 colonies per 1 loop of blood	Blood taken 2 hrs. before death. No pulmo- nary edema. Respi- rations very rapid, but became slow and ster- torous before death. Heart sounds audible for 3 min. after res- piration stopped. Average capacity of 6 normal individuals and 6 convalescents, 19.57 per cent.

ment of the severe terminal symptoms and of the bacteremia, or, as in case 8, the rapid spread of the process in the lung. Thus in almost all cases of pneumonia running an uneventful course, the oxygen content of the blood remains within normal limits, but in

the majority of fatal cases, the terminal stage is marked by a progressive decrease in the oxygen content of the blood.

Since the pneumococcus shows an intense avidity for oxygen, it was possible that the mere presence of the organism in the blood was sufficient to explain the decrease in the oxygen content of the blood. It was therefore important to determine whether there was any actual change in the hemoglobin molecule which interfered with its taking up oxygen normally. For this purpose samples of blood were shaken in air until saturated with oxygen, and then the total combined oxygen was determined. Six specimens of blood from five convalescent pneumonia cases and one normal individual gave an average oxygen capacity of 19.57 per cent. In eight of the fatal pneumonia cases, similar observations were made. Case 11, which, as has been said, died very suddenly, had a normal oxygen content of the blood two hours before death and showed at the same time a normal oxygen-combining power. On the other hand, all the cases which had a low oxygen content showed also a low oxygen-combining power in specimens of blood taken just before or, as nearly as possible, at the time of death. The progressive nature of this fall in the oxygen-combining power of the blood was shown in four out of five cases, in which more than one observation was made during the terminal stage. In the fifth case the blood had as low a capacity twenty-four hours before death as it did at death. The amount of oxygen given off by fully saturated samples of blood taken at death varied considerably from case to case. The highest was 16.84 per cent., the lowest was 7.87 per cent. Four were between 13.23 and 15.09 per cent. In general, then, although the fall in the oxygen-combining power of the blood is slightly less marked than is the fall in the actual oxygen content of the venous blood, the two run parallel to one another.

The blood after death was very dark and often of a brownish tint. It coagulated much more slowly than normally. The serum was alkaline to litmus in the only case tested. Spectroscopic examination in several cases showed only the absorption bands of oxyhemoglobin or hemoglobin. Methemoglobin was not found. Hemoglobin estimations on several cases ruled out the possibility of the low oxygen-combining power being due to anemia.

The explanation of the striking changes shown in the oxygen content and in the oxygen-combining power of the blood in the terminal stage of fatal cases of pneumonia is to be found by turning to experimental studies. Butterfield and Peabody¹⁰ have shown that when the pneumococcus grows in a mixture containing red blood cells, there is a gradual conversion of the hemoglobin into methemoglobin, and coincident with this a decrease in the extent to which the blood can take up oxygen. There is, moreover, a marked fall in the oxygen-combining power of such mixtures, even when no methemoglobin absorption bands can be distinguished spectroscopically. Peabody¹¹ has also found that similar changes take place in rabbits with a severe pneumococcic bacteremia. When the infection in rabbits becomes advanced, there is a gradual fall in the oxygen content of the arterial blood and also in the oxygen-combining power of the blood. The fall both in the oxygen content of the arterial blood and in the oxygen-combining power is a gradually progressive one up to the time of death. At the time of death the combining power is usually only between 60 and 75 per cent. of its original value. In some animals the oxygen content of the blood falls more rapidly than the oxygen-combining power, so that the arterial blood is less completely saturated with oxygen than under normal conditions. The suggestion was thus made that, besides a fall in the actual combining power of the blood, there is also a slowing in the rate of the reaction.

Conditions similar to those produced experimentally in rabbits are found in the fatal cases in man. In both there is a progressive decrease in the oxygen content of the blood. This has only been demonstrated for the venous blood of man, but it occurs in the arterial blood of rabbits and probably also in the arterial blood of man. In man and in the rabbit there is also a progressive diminution in the oxygen-combining power of the blood, reaching its minimum at the time of death. In the experimental infections and usually in the fatal cases in man there is a severe bacteremia. The conditions in man and in the rabbit are so similar to those in the test-tube that it seems logical to explain all three in the same way

¹⁰ Butterfield, E. E., and Peabody, F. W., *loc. cit.*

¹¹ Peabody, F. W., *Jour. Exper. Med.*, 1913, xviii, 1.

and to account for the changes in the blood by the growth of the pneumococcus. In solutions of laked hemoglobin the pneumococcus produces amounts of methemoglobin that are easily found with the spectroscope. In mixtures of unlaked red cells the change goes on much more slowly and is less complete. In the human body and in experimental animals death occurs before enough methemoglobin has been formed to be visible spectroscopically, but the falling off of the oxygen-combining power of the blood indicates that such a change is probably taking place. Case 8 never had any demonstrable bacteremia, but it has been shown that if a bouillon culture of the pneumococcus be passed through a Berkefeld filter, the filtrate will have the same effect on blood as does the organism itself. It may thus be assumed, with comparative certainty, that the changes in the blood in this case are due to the passage into the blood stream of the products of the growth of the organism in the lungs. Case 11, the only fatal case not showing changes in the oxygen of the blood, died suddenly. It is possible that had he lived longer, the organisms would have developed and produced the characteristic changes in the blood.

The progressive fall in the oxygen content and in the oxygen-combining capacity of the blood in fatal cases of pneumonia is thus undoubtedly the direct result of the growth of the pneumococcus. An interference with the respiratory exchange of gases in the lungs, or a circulatory effect could not explain the conditions, for the low oxygen was associated with a low carbon dioxide content of the blood,¹² and any condition which interfered with the taking up of oxygen would also interfere with the excretion of carbon dioxide. Moreover, an abnormal respiratory exchange would not affect the oxygen-combining power of the blood. The same change is also present in the arterial blood of rabbits infected with the pneumococcus, and in these animals the lungs are not involved. In how far the changes produced in the blood are the actual cause of death, it is impossible to say, but that they are a factor, and probably an important one, can hardly be denied. The terminal symptoms,—toxemia, cardiac failure, polypnea, pulmonary edema, and cyanosis,—run parallel to the changes in the blood and may well

¹² Peabody, F. W., *loc. cit.*, 1912, xvi, 701.

be due in part to a deficient oxygenation dependent on these changes. It is interesting that in case 8 the temporary rise in the oxygen values was associated with a marked clinical improvement. While not attempting to assign a rôle that is all important to the changes in the blood, it would seem correct to consider that in many cases of pneumonia the terminal symptoms and death are in part dependent on the changes produced in the hemoglobin molecule by the growth of the pneumococcus.

CONCLUSIONS.

In most cases of uncomplicated lobar pneumonia the decrease of respiratory surface is completely compensated for, and the oxygen content of the blood is within normal limits. Occasional cases of uncomplicated pneumonia have an oxygen content of the venous blood which is below normal. In the two cases reported here, this was associated with a carbon dioxide content of the blood which was higher than normally, and the condition was apparently due to an interference with the respiratory exchange of gases.

In the terminal stage of the fatal cases of pneumonia in which death does not occur with great suddenness, there is often a progressive diminution in the oxygen content of the blood. Synchronous with this is a progressive decrease in the oxygen-combining capacity of the blood. These changes are usually seen in patients in whom an intense bacteremia has developed and are analogous to those found in the arterial blood of infected rabbits, and to those resulting from the growth of the pneumococcus in blood *in vitro*. In all three conditions there is probably a change of oxyhemoglobin to methemoglobin. This change of the hemoglobin molecule, so that it no longer takes up and gives off oxygen readily, is probably a factor in the immediate cause of death in many cases of pneumonia.

A STUDY OF THE ADDITION OF CHOLESTERIN TO THE ALCOHOLIC EXTRACTS OF TISSUES USED FOR ANTIGENS IN THE WASSERMANN REACTION.*

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The non-specific character of the so called antigen used in the Wassermann reaction has permitted a wide variation in the selection of material from which it has been prepared. Consequently, the reaction has yielded varying results in the hands of different workers. It is the object of this communication to compare the results obtained with several of the more extensively used antigens.

In spite of much experimentation no constant antigen has been adopted by a large group of workers. Practically all have agreed that the alcoholic extracts of livers of syphilitic fetuses are the best. The same method of preparation from different fetal livers will, however, yield reagents of widely different qualities. Many of them are hemolytic, others contain an excess of non-specific anticomplementary substances, and others have only feeble fixing power, so that probably not half the preparations made from these tissues can be used. Frequently very good reagents can be prepared by simple alcohol extraction of the livers of non-syphilitic fetuses or stillborn babies. The irregularity in the strength of antigens prepared from livers, as well as the difficulty in obtaining fetal livers, has led many to use alcoholic extracts of hearts. For this purpose, human, beef, and guinea pig hearts have been mostly employed. Lesser (1) has used saline emulsions of the residue obtained by extracting dried human hearts with ether, and this has been found by Dembowski (2) to yield a better antigen than alcoholic extraction. Such an ether extract is now on the market.

In searching for the active substance, Noguchi (3) concluded that a pure lipid which consisted of the ether-soluble, acetone-insoluble portion of an alcoholic extract of either hearts or livers was the most reliable antigen. His method of preparation yielded, however, substances of widely different properties, so that each extract had to be thoroughly tested before it was adopted as a standard reagent. Browning and McKenzie (4) recommended a slightly different technique for the preparation of the pure lecithin. They found that a lecithin prepared from beef liver had good antigenic properties, though some-

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what less active than the crude alcoholic extract from the same organ. The addition of cholesterol to the lecithin produced an antigen which was superior to either the lecithin or the crude alcoholic extract. These authors claimed that if a given serum absorbed more complement in the presence of a lecithin-cholesterol mixture than in the presence of lecithin alone, the serum was surely from a syphilitic subject. Cholesterol alone had practically no antigenic action. Sachs (5) applied the method of fortifying with cholesterol to crude alcoholic extracts of normal hearts. He extracted beef hearts with five volumes of alcohol, and to 5 c.c. of this extract he added 0.3, 0.4, or 0.5 c.c. of a 1 per cent. alcoholic solution of cholesterol. The optimum amount of cholesterol was determined by comparing the different mixtures with a standard antigen. Often alcohol was added to the mixture, for Sachs, as well as Browning and McKenzie, has claimed that the addition of a certain amount of alcohol will increase the activity of an antigen. Sachs states that this activity is due not only to the concentration of the lipoids, but to their relative proportions. He reports that the cholesterol-fortified extract of beef heart was as strong an antigen as the best alcoholic extract of syphilitic liver.

McIntosh and Fildes (6) found that an antigen composed of five parts of alcoholic extract of human heart plus four parts of 1 per cent. alcoholic solution of cholesterol was much superior to either an alcoholic extract of normal heart, an alcoholic extract of syphilitic liver, or the lecithin-cholesterol mixture of Browning. More important still, they determined that antigens prepared in this manner from twenty-four different human hearts had all practically the same antigenic and anticomplementary properties. Their results seem to indicate that the standard antigen should be a mixture of heart extract and cholesterol.

Since the optimum amount of cholesterol seems never to have been determined, it seemed desirable to ascertain it, and, in addition, to compare the cholesterol-fortified extracts from hearts and livers of different animals. The present communication is a report upon this aspect of the subject.

METHODS.

A slightly modified Wassermann technique was used. All quantities of the various reagents were one half the amounts originally described by Wassermann, Neisser, and Bruck (7).

Hemolytic System.—0.5 of a cubic centimeter of a 5 per cent. suspension of sheep cells was used as a standard. The rabbit serum anti-sheep amboceptor was standardized with a complement of known strength. By using diminishing quantities of the amboceptor with one previously determined unit of complement, the minimum hemolytic dose of the amboceptor was determined. The amboceptor was then diluted, so that 0.25 of a cubic centimeter contained one unit of amboceptor. It was found to be important to determine

this amount accurately, because comparatively slight variations in the amount of amboceptor used cause variation in the amount of complement necessary. Protocol I shows this point clearly.

PROTOCOL I.

Method of Determination of the Hemolytic Unit of Amboceptor.

Tube.	Amount of amboceptor dilution.	Complement, guinea pig serum 10 per cent.	Sheep cells 5 per cent.		Dilution of amboceptor.			
					1 : 250	1 : 300	1 : 350	1 : 400
1	0.5 c.c.	0.5 c.c.	0.5 c.c.	All made up to 2.5 c.c. Incubation 1 hr. at 37° C.	C.H. ¹	C.H.	C.H.	C.H.
2	0.4 c.c.	0.5 c.c.	0.5 c.c.		C.H.	C.H.	C.H.	C.H.
3	0.3 c.c.	0.5 c.c.	0.5 c.c.		C.H.	C.H.	C.H.	C.H.
4	0.25 c.c.	0.5 c.c.	0.5 c.c.		C.H.	C.H.	C.H.	C.H.
5	0.2 c.c.	0.5 c.c.	0.5 c.c.		C.H.	C.H.	C.H.	C.H.
6	0.1 c.c.	0.5 c.c.	0.5 c.c.		C.H.	N.C.H.	N.C.H.	Mod. H.
7	0.25 c.c.	0.4 c.c.	0.5 c.c.		C.H.	C.H.	C.H.	C.H.
8	0.25 c.c.	0.3 c.c.	0.5 c.c.		N.C.H.	C.H.	C.H.	C.H.
9	0.25 c.c.	0.25 c.c.	0.5 c.c.		Mod. H.	N.C.H.	C.H.	N.C.H.
10	0.25 c.c.	0.2 c.c.	0.5 c.c.		Sl.H.	N.C.H.	N.C.H.	Sl.H.
11	0.25 c.c.	0.1 c.c.	0.5 c.c.		Sl.H.	Mod. H.	Mod. H.	Sl.H.
12	0.5 c.c.	—	—		0			
13	—	0.5 c.c.	—		0			
14	—	—	0.5 c.c.		0			

It had been previously determined that 0.0008 of a cubic centimeter of this amboceptor was the minimum hemolytic dose with one unit of complement. By working with dilutions so arranged that 0.25 of a cubic centimeter of dilution contained 0.0008 of a cubic centimeter of serum, another dilution slightly more, and a third slightly less than this amount, it was found that 1 to 350 was the proper dilution of amboceptor. This titration was repeated with several specimens of pooled complement until a constant optimum

¹ C.H. = complete hemolysis; N.C.H. = nearly complete hemolysis; Mod.H. = moderate hemolysis; Sl.H. = slight hemolysis; 0 = no hemolysis.

was determined. Two units of amboceptor diluted to 0.5 of a cubic centimeter with saline were used to sensitize each 0.5 of a cubic centimeter of the 5 per cent. cell suspension.

Complement.—The strength of the complement was determined each day. The previously determined optimum dilution of amboceptor was titrated against varying amounts of 10 per cent. complement (as shown in dilution 1 to 350, protocol I), and thus the minimum hemolytic dose of complement determined. The guinea pig serum was then diluted so that 0.5 of a cubic centimeter of the dilution represented two complement units. If hemolysis did not occur in tube 7 or went beyond tube 11, new complement was obtained. By accurately titrating the complement in this manner, the results of the fixation reactions from day to day are quite comparable.

Human Serum.—0.1 of a cubic centimeter of human serum inactivated one half hour at 56° C. was used in the test-tube; 0.2 of a cubic centimeter of the same serum was used in the serum anti-complementary control tube. The human serum was first pipetted into the tubes, then 0.4 of a cubic centimeter of saline was added to the test-tube, and 0.8 of a cubic centimeter of saline to the anticomplementary control tube. The complement was then added to all the tubes, and antigen to the test-tube. The volume of liquid in all the tubes was thus 1.5 cubic centimeters. Incubation was carried on in the water bath at 37° C. for one hour. The cells which had been sensitized by incubating 0.5 of a cubic centimeter of 5 per cent. cells with two amboceptor units for one half hour were then added to all the tubes, and incubation was carried on for another hour. Controls with positive serum, negative serum, and with antigen without serum were always employed.

Antigens.—Alcoholic extracts of the various organs were prepared as follows: The tissue, free from gross fat, was finely minced, weighed, and placed in absolute alcohol (Kahlbaum) in the proportion of one gram of tissue to ten cubic centimeters of alcohol. Extraction was carried on in the incubator at 37° C. for two to three weeks, the bottles being shaken daily. The extract was cooled to room temperature, filtered, and kept at room temperature as a stock solution. If a precipitate formed upon standing, the extract was

refiltered. The extraction was carried on in the incubator instead of by shaking because many laboratories are not equipped with a shaking machine, and it seemed desirable to adopt a technique for preparation of antigen which could be easily repeated.

The tissues selected for study were three human hearts, one beef heart, guinea pig hearts, guinea pig livers, and two non-syphilitic fetal livers. The guinea pig heart extract was made from a number of hearts, which were removed each day from the animals that had been killed for complement, weighed, minced, and placed with the requisite amount of alcohol in the common stock. This extract represented, therefore, the average extract of guinea pig hearts.

THE EFFECT OF VARYING CONCENTRATION OF CHOLESTERIN IN THE ALCOHOLIC TISSUE EXTRACT.

For this study four tissue extracts and a saturated alcoholic solution of a pure lipid from fetal livers were used. The tissues represented in the different extracts were human heart, beef heart, guinea pig liver, and fetal liver. Saturated cholesterin solutions in the alcoholic extracts were prepared by adding known quantities of cholesterin to 100 cubic centimeters of the alcoholic extract, shaking for several hours, and then allowing to stand at 37° C. over night. Upon cooling to room temperature, the excess of cholesterin precipitated out. The amount of this precipitate was determined by filtering through weighed filters, drying and weighing the precipitate. The difference between the amount of cholesterin originally added and the weight of the filtrate showed the amount of cholesterin taken up by each extract. The amount of cholesterin taken up by the different crude extracts varied between 0.72 and 0.9 of a gram per 100 cubic centimeters. The exact amount is given in table I. The alcoholic solution of pure lipid took up practically double this amount of cholesterin. Three fourths, one half, and one fourth saturated solutions were made by adding proper amounts of the original extract to the cholesterin-saturated extract.

Emulsions of the various dilutions were made by adding the extracts drop by drop, with constant shaking, to normal saline in the proportion of one part of the extract to five parts of saline, thus making a 1 in 6 emulsion. The emulsions were freshly prepared

each day in the same way. Sachs and Rondoni (8) showed that variations in the manner of mixing an alcoholic extract and saline varied the fixing power of the resulting mixture. Slow mixing of the alcoholic extract with the saline produced a heavy emulsion; rapid mixing, a colloidal suspension. The emulsion was more anticomplementary and fixed complement in smaller amounts in the presence of syphilitic serum. McIntosh and Fildes showed, however, that either slow or rapid mixing of their cholesterin-heart extract produced milky emulsions with equal fixing strength. We have repeated and confirmed this observation. If the extract is added slowly to the saline, a heavy emulsion forms at once; if added rapidly, the suspension is more opalescent at first, but upon standing becomes milky. After standing for one hour, both emulsions were tested for fixing power by using constant quantities of antigen against diminishing quantities of syphilitic serum. The point of disappearance of complete fixation was the same with both emulsions, thus proving that they had equal antigenic values.

With a constant method of emulsifying the antigen, the density of the emulsion was in direct proportion to the amount of cholesterin which had been added to the extract. In all instances, emulsion A (table I) was the heaviest, while emulsion E was opalescent.

The various emulsions from one extract were all tested for one property at the same time. The anticomplementary dose was first determined against two units of complement. This was done by increasing the amount of emulsion by steps of 0.1 of a cubic centimeter from 0.5 to 1.5 of a cubic centimeter. The addition of cholesterin increased the anticomplementary action of the extracts only slightly. With the heavier emulsions, the anticomplementary action was slightly more marked, but it began at practically the same point with all the emulsions of a given extract. The anticomplementary action of the heart extracts began at 1.2 cubic centimeters of the 1 in 6 emulsion, and at 1.4 cubic centimeters of the liver extracts.

The point at which fixation began with 0.1 of a cubic centimeter of serum from non-syphilitic patients was next determined. Serum from the same patients was used as much as possible with the various sets of extracts. The results of these experiments are summarized in table I and will be discussed later. Lastly, the smallest

amount of antigen which gave complete fixation with one fixing unit of syphilitic serum was determined. This fixing unit was established by titrating diminishing quantities of the serum with our old standard antigen. The amount of serum which just gave complete fixation was called a fixing unit. This amount was then diluted to 0.5 of a cubic centimeter with saline and tested against diminishing amounts of the various emulsions of the extract. The serum from both early and late stages of syphilis was tested with each set of emulsions.

Table I gives the point at which anticomplementary action of the emulsions began, the point of beginning fixation with non-syphilitic serum, and the point of complete fixation with syphilitic serum. In addition, the ratio of the fixing dose to the anticomplementary dose is given in per cent., or, in other words, the first two percentage columns indicate the proportion of the anticomplementary dose of antigen which showed beginning fixation with non-syphilitic serum, and the second two columns the proportion of anticomplementary dose which gave complete fixation with syphilitic serum. With the completely saturated, and three fourths and one half saturated cholesterolin in the heart extracts, one unit of serum from patients with secondary syphilis gave complete fixation at points varying between 6 and 16 per cent. of the anticomplementary dose. One unit of serum from patients with late syphilis or tabes gave complete fixation with much smaller amounts of antigen; viz. between 1.6 and 6.6 per cent. With the one fourth saturation of cholesterolin the point of fixation with negative serum rose between 15 and 30 per cent., while the curve with the serum from secondary syphilis rose from 8 per cent. for one half saturation to more than 60 per cent. for one fourth saturation of cholesterolin. This falling off was less marked with serum from tertiary syphilis or tabes. The cholesterolinized fetal liver extract reacted with negative serum and secondary syphilitic serum in much the same manner as did the heart extract, but with the serum from tabes a striking difference is noted. Fixation with this serum did not go to nearly so low a point with any of the liver extracts as it did with those from the hearts.² This would

² The results shown in table I were confirmed several times. Only examples with each class of serum, viz., non-syphilitic, early syphilitic, and late syphilitic, are given in order not to increase unnecessarily the size of the table.

TABLE I.

Alcoholic extract of	Human heart.*					Beef heart.				
	Complete.	1/2	1/4	1/8	o	Complete.	1/2	1/4	1/8	o
Saturation of cholesterol										
Per cent. of cholesterol added.	0.9	0.67	0.45	0.22	o	0.72	0.54	0.36	0.18	o
Emulsion.	A	B	C	D	E	A	B	C	D	E
Anticomplementary dose in c.c.	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Beginning fixation with 0.1 c.c. negative serum.	0.5	0.5	0.5	0.8	1	0.6	0.5	0.6	1.0	1.1
Ratio of point of fixation with negative serum to anticomplementary dose in per cent.	41	41	41	66	80	50	41	51	80	90
Beginning fixation with 0.1 c.c. negative serum.	0.6	0.5	0.6	0.8	0.8	0.5	0.5	0.6	1.0	1.0
Ratio of point of fixation with negative serum to anticomplementary dose, in per cent.	50	41	50	66	80	41	41	50	80	80
Complete fixation with serum of early syphilis, in c.c.	0.2	0.2	0.1	0.7	0.9	0.2	0.2	0.08	0.7	0.8
Ratio of point of fixation with fixing unit of serum from early syphilis to anticomplementary dose, in per cent.	16	16	8	60	80	16	16	6.6	60	80
Complete fixation with serum of late syphilis, in c.c.	0.06	0.06	0.08	0.1	0.7	0.02	0.02	0.04	0.1	0.7
Ratio of point of fixation with fixing unit of serum from late syphilis to anticomplementary dose, in per cent.	5	5	6.6	8.5	60	1.6	1.6	3.3	8.3	60

* Unit = 0.006 c.c.

* Unit = 0.007 c.c.

* Unit = 0.1 c.c.

Alcoholic extract of	G guinea pig liver.					Fetal liver.				
	Complete.	%	1/2	1/4	0	Complete.	%	1/2	1/4	0
Saturation of cholesterol.										
Per cent. of cholesterol added.	0.8	0.6	0.4	0.2	0	0.9	0.67	0.45	0.22	0
Diagnosis										
Emulsion	A	B	C	D	E	A	B	C	D	E
Anticomplementary dose in c.c.	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4
Beginning fixation with 0.1 c.c. negative serum	0.5	0.6	0.8	1.2	1.2	0.6	0.6	0.8	0.8	1.2
Pneumonia										
Ratio of point of fixation with negative serum to anticomplementary dose, in per cent.	35	43	57	90	90	43	43	57	57	90
Beginning fixation with 0.1 c.c. negative serum	0.5	0.6	0.9	1.2	1.2	—	—	—	—	—
Endocarditis										
Ratio of point of fixation with negative serum to anticomplementary dose, in per cent.	35	43	64	90	90	—	—	—	—	—
Complete fixation with serum of early syphilis, in c.c.	0.2	0.3	0.6	1.1	1.2	0.2	0.2	0.1	0.2	0.5
Secondary active ¹										
Ratio of point of fixation with fixing unit of serum from early syphilis to anticomplementary dose, in per cent.	14	21	43	80	90	14	14	7	14	50
Complete fixation with serum of late syphilis, in c.c.	0.2	0.2	0.6	0.6	1.0	0.5	0.5	0.5	0.5	1.0
Tertiary active ¹										
Ratio of point of fixation with fixing unit of serum from late syphilis to anticomplementary dose, in per cent.	14	14	43	43	71	35	35	35	35	70

* Unit = 0.013 c.c.

† Unit = 0.1 c.c.

seem to indicate that cholesterin-heart extracts are specially active with the serum of patients with long standing syphilis. This view was substantiated in the second part of the present work.

The guinea pig liver extract acted somewhat differently than did the other extracts. The point of fixation with both normal and syphilitic serum fell off steadily as the concentration of cholesterin was diminished. With the simple alcoholic extracts of all the tissues (emulsion E) which contained no added cholesterin, the point of fixation with syphilitic serum approached so close to the point of fixation with negative serum and to the anticomplementary dose that for diagnostic purposes this form of emulsion had practically little value.

The result of the addition of cholesterin to a saturated alcoholic solution of pure lipoid prepared from fetal livers after Noguchi's method is not tabulated. These mixtures produced very heavy emulsions in salt solution. The point of fixation with normal serum was so close to the point of fixation with syphilitic serum that this form of extract could not be safely employed.

The results obtained with the two heart extracts have several points in common. Emulsions A, B, and C gave beginning fixation with negative serum at points between 40 and 50 per cent. of the anticomplementary dose. The fixing points with negative serum and with one unit of serum from secondary syphilis approached one another with emulsion D (one fourth saturation with cholesterin), and were practically the same with the simple alcoholic extract (emulsion E). With both heart extracts, as well as with the fetal liver extract, less of emulsion C than of emulsion A or B was required to give complete fixation with one unit of serum from early syphilis. The fixing point of all the cholesterin-heart extracts was low with the serum from late syphilis. The cholesterin fetal liver extracts gave fixation with normal serum and with serum from early syphilis in much the same amounts as did the cholesterin-heart extracts, but their fixing power with serum from late syphilis was much inferior to their fixing power with serum from secondary syphilis. The difference in the heart and liver extracts cannot be due to their original cholesterin content, because it was found that the heart extract contained 0.008 per cent. free cholesterin and 0.004

per cent. combined cholesterin, or a total of 0.012 per cent., while the liver extract contained 0.134 per cent. free cholesterin. If the cholesterin were alone responsible for the superiority of an extract, one would expect that the liver extract, which contained ten times as much cholesterin as the heart extract, would be the better, but this is not the case. It seems, therefore, that some other lipoid must be responsible for the superiority of the heart extract. The cholesterin guinea pig liver extracts were inferior to the other extracts, and the results obtained with them, compared with the results with two heart extracts or fetal liver extract, were so irregular that they were discarded in the subsequent work.

In the majority of trials, emulsion C was the most sensitive with serum from secondary syphilis, and was practically as sensitive as the stronger emulsions with the serum from late syphilis. In addition, its tendency to give non-specific fixation was somewhat less marked than were emulsions A and B. As previously mentioned, the density of the emulsions was in direct proportion to the amount of cholesterin it contained. Emulsions A and B were very milky, so that it was difficult to make immediate readings of the degree of hemolysis. As a result, the degree of partial hemolysis could not be determined until the following morning, when the non-hemolyzed cells had settled to the bottom of the tube. Emulsion C was not so milky, and with it immediate readings could be made with a fair degree of accuracy. In practically all respects emulsion C was superior to any of the other emulsions. Emulsion C consisted of equal parts of the saturated cholesterin extract and of the original extract. The amount of the added cholesterin in half saturation was between 0.36 and 0.45 per cent., or an average of slightly more than 0.4 per cent. The results of the first part of the work indicate that about 0.4 per cent. cholesterin is the optimum amount to add to crude alcoholic extracts of either hearts or human fetal livers. The effect of adding the cholesterin previously dissolved in alcohol was studied. Forty cubic centimeters of a 1 per cent. alcoholic solution of cholesterin were added to sixty cubic centimeters of the stock alcoholic extract of human heart C. The resulting mixture, therefore, contained 0.4 per cent. cholesterin. This was compared with the same alcoholic heart extract to which 0.4 per cent. cholesterin

had been added directly. The anticomplementary dose of 1 in 6 emulsions of the extracts was found to be 1.2 cubic centimeters for the cholesterin-heart extract and 1.1 cubic centimeters for the alcoholic cholesterin plus alcoholic heart extract. The cholesterin-heart extract gave slightly better fixation with syphilitic serum than did the alcoholic cholesterin plus alcoholic heart extract. The results of these experiments indicate that it was better to dissolve the cholesterin directly in the alcoholic extract of the heart. McIntosh and Fildes' cholesterin-heart extract was made by adding four parts of 1 per cent. alcoholic solution of cholesterin to five parts of heart extract which yielded a mixture containing 0.44 per cent. cholesterin; so the results obtained with their antigen and ours should be nearly comparable.

COMPARISON OF DIFFERENT EXTRACTS WITH THE SAME AMOUNT OF CHOLESTERIN ADDED TO EACH.

After it was decided that 0.4 per cent. cholesterin was the optimum amount to add to crude alcoholic extracts of the various organs, this amount of cholesterin was added to all the extracts at hand, and the resulting mixtures were compared for antigenic value. For this comparison we used extracts from three human hearts (C, D, and T), one beef heart (B), guinea pig hearts (G), and two fetal livers (L and K). As a standard for comparison we used three different antigens. One of them, antigen 29, was an alcoholic extract of fetal liver to which 0.25 per cent. cholesterin had been added. This had been in use for some time and had proven satisfactory according to our old standards. It was more sensitive than the antigens used in most other laboratories.⁸ The other standard antigens were, first, an ether extract of dried human heart.⁹ About two thirds of the sera were tested with this antigen. The remainder were tested with an alcoholic extract of syphilitic liver.¹⁰ The combined results with these two antigens show that they were

⁸ Dr. Jagle found it more sensitive than a standard antigen that was prepared in Sachs' laboratory in Frankfurt (personal communication).

⁹ This was prepared by Tanenzen-Apotheke-R. Maertens, Berlin, after Fritz Lesser's formula.

¹⁰ This was prepared by Gans, Frankfurt a/M, and standardized by Wassermann and Meier.

somewhat less active than our antigen 29. The 0.4 per cent. cholesterin heart and liver extracts were first tested for anticomplementary action; 1 in 6 emulsions were prepared and tested against two units of complement. It was found that the heart extracts were anticomplementary at about the same point, 1.2 cubic centimeters, and the liver extracts at 1.3 cubic centimeters. Three different strengths of emulsions of each extract in saline were tested for fixing power. These were 1 in 6, 1 in 10, and 1 in 30. 0.5 of a cubic centimeter of the 1 in 6 emulsion is about 40 per cent. of the anticomplementary dose. This amount was shown in the first part of the work to be the largest quantity that would not give non-specific fixation. 0.5 of a cubic centimeter of 1 in 10 emulsion is 25 per cent., and the same amount of 1 in 30 is 8 per cent. of the anticomplementary dose. The first twenty-six sera were tested with all the emulsions. This number was sufficient to demonstrate that the cholesterin-liver extracts were not as sensitive as cholesterin-heart extracts, and that the three human heart extracts had practically the same fixing power. The comparison of two human heart extracts (D and T), and two fetal liver extracts (L and K) is given in the second part of table II. The remainder of the tests were carried on with cholesterinized extracts from one human heart (C), the beef heart (B), and the guinea pig hearts (G), as well as with the standard antigens 29, Lesser, and Wassermann. Most of the sera were from patients who had received more or less treatment with salvarsan and mercury. The few sera from untreated patients, both secondary and tertiary, gave complete fixation with all strengths of all the antigens. For purposes of comparison, the cases are divided into early and late syphilis. The early cases were all within three years from the time of infection,—most of them in the first year. The late cases were in the fourth year or later, and include cases of tabes dorsalis. There were thirty-five early cases and thirty-six late ones.

The results of the entire series are given in table II. The 1 in 6 emulsions of cholesterin-heart extracts gave a surprising number of strong positive reactions,—nearly three times as many as were obtained with the old standard antigens. As will be shown later, this 1 in 6 emulsion was probably too strong. The 1 in 10 emulsions

TABLE II.¹¹

Strength of emulsion.		1:6			1:10			1:30			Lesser Wasserm.			Total.			18 cases			8 cases			25			1:10			1:30										
Extract.		C.	B.	G.	C.	B.	G.	C.	B.	G.	C.	B.	G.	D.	T.	L.	K.	D.	T.	L.	K.	D.	T.	L.	K.	D.	T.	L.	K.										
++	No.	20	18	21	10	8	12	8	8	8	4	1	5	7													13	13	7	7	6	7	4	5	6	6	3	4	
	Per cent.	57	51	60	29	23	34	23	23	23			14	20													72	72	40	40	33	40	22	28	33	33	17	22	
	+	No.	5	5	4	7	4	6	1	1	2	1	1	2	1													2	2	4	5	1	3	2	1			1	1
		Per cent.	14	14	11	20	11	17	3	3	6			6	3													11	11	22	28	5	17	11	5			5	5
	±	No.	4	2	3	1	5	1	3	1	2	1	1	2	5													1				1	1	1	1			2	1
		Per cent.	11	6	9	3	14	3	9	3	6			6	14													5				5	5	5	5			11	5
	± (?)	No.	1	4	3	3	3	4	1	1	1	5		5	7													2	3	3	4	4	2	2	3	1	1		
		Per cent.	3	11	9	9	9	11	3	3	3			14	20													11	17	22	22	11	11	17	5	5			
	-	No.	5	6	4	14	15	12	22	24	22	11	10	21	14															4	1	6	5	9	8	11	9	13	13
		Per cent.	14	17	11	40	43	34	63	68	63			60	40															22	5	33	28	50	44	62	50	72	72
++	No.	23	19	23	18	17	18	13	13	14	2	5	7	6													5	5	5	5	5	5	3	3	5	5	2	2	
	Per cent.	61	53	61	50	47	50	36	36	40			20	17													63	63	63	63	63	37	37	63	63	25	25		
	+	No.	5	8	5	5	2	5	2	2	2	2	3	5	3													1	1									2	2
		Per cent.	14	22	14	14	6	14	6	6	6			14	8													12	12									25	25
	±	No.	2	3	2	1	2	2	1	1				1	3													1	1										
		Per cent.	6	9	6	3	6	6	3	3				3	9													12	12	12	12								

Early syphilis 35 cases	++	No.	20	18	21	10	8	12	8	8	8	4	1	5	7													13	13	7	7	6	7	4	5	6	6	3	4
		Per cent.	57	51	60	29	23	34	23	23	23			14	20													72	72	40	40	33	40	22	28	33	33	17	22
	+	No.	5	5	4	7	4	6	1	1	2	1	1	2	1													2	2	4	5	1	3	2	1			1	1
		Per cent.	14	14	11	20	11	17	3	3	6			6	3													11	11	22	28	5	17	11	5			5	5
	±	No.	4	2	3	1	5	1	3	1	2	1	1	2	5													1				1	1	1	1			2	1
		Per cent.	11	6	9	3	14	3	9	3	6			6	14													5				5	5	5	5			11	5
	± (?)	No.	1	4	3	3	3	4	1	1	1	5		5	7													2	3	3	4	4	2	2	3	1	1		
		Per cent.	3	11	9	9	9	11	3	3	3			14	20													11	17	22	22	11	11	17	5	5			
	-	No.	5	6	4	14	15	12	22	24	22	11	10	21	14															4	1	6	5	9	8	11	9	13	13
		Per cent.	14	17	11	40	43	34	63	68	63			60	40															22	5	33	28	50	44	62	50	72	72
Late syphilis 36 cases	++	No.	23	19	23	18	17	18	13	13	14	2	5	7	6													5	5	5	5	5	5	3	3	5	5	2	2
		Per cent.	61	53	61	50	47	50	36	36	40			20	17													63	63	63	63	63	37	37	63	63	25	25	
	+	No.	5	8	5	5	2	5	2	2	2	2	3	5	3													1	1									2	2
		Per cent.	14	22	14	14	6	14	6	6	6			14	8													12	12									25	25
	±	No.	2	3	2	1	2	2	1	1				1	3													1	1										
		Per cent.	6	9	6	3	6	6	3	3				3	9													12	12	12	12								

¹¹ C, D, T = cholesterol-human heart extract; B = cholesterol-beef heart extract; G = cholesterol-guinea pig heart extract; L, K = cholesterol-fetal liver extract; Lesser = ether extract of human heart; Wassermann = alcoholic extract of syphilitic liver; 20 = cholesterol fetal liver extract (standard); ++ = complete inhibition of hemolysis; + = about 50 per cent. hemolysis; ± = about 75 per cent. hemolysis; ± (?) = nearly complete hemolysis; — = complete hemolysis.

gave twice the number of strong positive reactions given by the old antigens. If we include + + and + reactions as strongly positive, the results are 57 per cent. with the human and guinea pig heart extracts, 45 per cent. with the beef heart, against 25 per cent. with antigen 29, and 27 per cent. for the combined results with the Lesser and Wassermann-Meier antigens. The 1 in 30 emulsions gave from 34 to 36 per cent. of strong positive reactions, which is an advantage of about 10 per cent. over the old antigens. Although the cholesterin-heart antigens were more sensitive in early syphilis, their superiority was more marked in late syphilis. With sera from early syphilis, the human heart antigen 1 in 10 gave 49 per cent. combined + + and + reactions, against 23 per cent. with antigen 29, and in late syphilis 64 per cent. of reactions of this strength with early syphilis and 42 per cent. with late syphilis. This increased sensitiveness with sera from late syphilis is a great advantage, for it is this class of cases in which the Wassermann reaction has such great diagnostic value.

The difference in cholesterin-heart and cholesterin-liver extracts with serum from early and late syphilis is well illustrated in the second half of the table. With serum from early syphilis and decreasing strength of antigens, that is, dilutions of 1 in 6, 1 in 10, and 1 in 30, the + + reactions with the best cholesterin-heart antigens were 72, 40, and 33 per cent., respectively, and with the cholesterin-liver antigen, 40, 28, and 22 per cent. In late syphilis the results with the cholesterin-liver antigens were 63, 37, and 25 per cent., while all dilutions of the heart-cholesterin antigens gave 63 per cent. positive reactions. Decreasing the strength of both kinds of antigen in secondary syphilis and of the cholesterin-liver antigen in tertiary syphilis decreased the number of positive reactions, while a similar diminution in strength of the cholesterin-heart antigen with sera from late syphilis had much less effect. This point was mentioned in the early part of the communication. A study of this rather striking difference between cholesterin-heart and cholesterin-liver antigens may throw some light on the nature of the Wassermann reaction. It should be emphasized that the sera were mostly from treated patients, so that the results do not indicate the percentage of positive reactions obtained with sera from early and

late untreated syphilis; all the sera from untreated patients gave strong positive reactions.

Human heart extracts were of practically the same strength. The guinea pig heart extract was very slightly stronger than the human heart extract. The beef heart extract, on the other hand, was decidedly inferior to the other heart extracts. The total ++ and + reactions with 1 in 10 emulsions were 57 per cent. with the human and guinea pig heart extracts, against 45 per cent. with beef heart extract. As all the cholesterin-heart extracts were superior to similar preparations from human fetal livers, our results indicate that human or guinea pig hearts are the best tissues from which to prepare alcoholic extracts for reinforcement with cholesterin.

Twenty-two sera from patients in whom syphilis could be excluded were tested with all strengths of the three heart antigens, as well as with the standard antigens. Sixteen of these were entirely negative, and of these sixteen, twelve were also negative with 0.7 of a cubic centimeter of the 1 in 6 emulsion. Six of the twenty-two sera gave some degree of positive reaction with all of the 1 in 6 emulsions, three of these were + and three others $\pm$ (?). None of the twenty-two sera gave any inhibition with 1 in 10 or 1 in 30 emulsions. We feel that an antigen that gives any degree of positive reactions with 27 per cent. of non-syphilitic sera is too sensitive for diagnostic purposes. As all the non-syphilitic sera reacted negatively with 1 in 10 emulsions, we believe that this dilution is not too sensitive. Emulsions of this strength were one fourth of the anticomplementary dose, which should be well within the limits necessary to avoid non-specific fixation. McIntosh and Fildes state that one third of the anticomplementary dose of their cholesterin-heart antigen gave no non-specific reactions. They disregarded slight degrees of inhibition, calling such reactions negative. In view of the fact that such a large proportion of the non-syphilitic sera which we tested gave negative reactions with 1 in 6 emulsions, and that 15 per cent. of the sera from treated syphilitic patients also gave negative reactions with this strength of extract, it may be that syphilitic patients who are under treatment should be treated until their sera give negative reactions with 1 in 6 emulsions. This point

TABLE III.

Antigen.	29					C. 1, 10					C. 1, 6						
	0.5 C.C.	0.4 C.C.	0.3 C.C.	0.2 C.C.	0.05 C.C.	0.5 C.C.	0.4 C.C.	0.3 C.C.	0.2 C.C.	0.1 C.C.	0.05 C.C.	0.5 C.C.	0.4 C.C.	0.3 C.C.	0.2 C.C.	0.1 C.C.	0.05 C.C.
Amount of cerebrospinal fluid.	++	++	++	++	—	++	++	++	++	++	—	++	++	++	++	++	—
Syphilis	++	++	++	++	—	++	++	++	++	++	—	++	++	++	++	++	—
	++	++	++	++	—	++	++	++	++	++	—	++	++	++	++	++	—
	++	++	++	++	—	++	++	++	++	++	—	++	++	++	++	++	—
	—	—	—	—	—	++	++	—	—	—	—	++	++	++	—	—	—
	++	++	++	++	—	++	++	++	++	++	—	++	++	++	++	++	—
Cerebral.....	—	—	—	—	—	++	++	++	++	—	—	++	++	++	—	—	—
Tabes.....	++	++	++	++	—	++	++	++	++	++	—	++	++	++	++	++	—
Tabes.....	±	—	—	—	—	++	++	++	±	—	—	++	++	++	±	—	—
Tabes.....	++	++	+	±	—	++	++	++	++	—	—	++	++	++	+	—	—
Tabes.....	++	++	++	++	—	++	++	++	++	±	—	++	++	++	++	±	—
Tabes.....	++	++	+	±	—	++	++	++	++	—	—	++	++	++	++	±	—
Tabo paralysis.....	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
Summary.....	++	9	9	6	5	2	1	12	11	9	6	1	12	12	7	5	1
	+	0	0	2	0	1	0	0	1	0	0	0	0	0	1	1	0
	±	1	0	1	2	0	0	0	2	0	0	0	0	1	2	1	1
	—	2	3	3	5	9	11	0	0	1	5	11	0	0	2	5	10

can be decided only after prolonged observation of a series of treated patients.

The results of the examination of twelve cerebrospinal fluids is given in table III. In addition, fifteen cerebrospinal fluids from well treated tabetics or from patients in earlier stages of syphilis were negative with all the antigens. It has been found that the use of larger quantities of spinal fluid than serum will give a high per cent. of positive reactions if the fluids are from patients with syphilitic disease of the central nervous system. The quantitative reaction in the cerebrospinal fluid permits accurate estimation of the point at which fixation begins. The slight anticomplementary action of spinal fluid as compared with blood serum makes it a most suitable reagent for comparison of antigens. Table III, in which are presented the results obtained with positively reacting fluids, shows that the heart-cholesterin antigens gave complete fixation with from 0.1 to 0.3 of a cubic centimeter less fluid than did antigen 29. More interesting is the comparison of the 1 in 10 and 1 in 6 emulsions of the heart-cholesterin antigen. Although both emulsions gave positive fixations with 0.5 of a cubic centimeter of all the fluids, with 0.3, 0.2, and 0.1 of a cubic centimeter of fluid there were more complete fixations with the 1 in 10 emulsions than with the 1 in 6. We are unable to explain this paradox of more fixations with an apparently weaker antigen. The absence of any marked anticomplementary action on the part of cerebrospinal fluid seems to warrant the conclusion that the 1 in 10 emulsion has as good a specific action as the stronger emulsion. Compared with the results which we obtained with blood sera, one might be justified in concluding that the larger per cent. of positive reactions obtained with 1 in 6 emulsions with the serum was due to the more marked anticomplementary action of emulsions of this strength. The results with the spinal fluid indicate that the 1 in 10 emulsion gives the maximum specific fixation. We know that the stronger emulsion is much more anticomplementary, so that the larger number of positive reactions given by it, both with syphilitic and non-syphilitic sera, could be easily explained by the summation of anticomplementary action of the blood serum and of the strong emulsion. This hypothesis cannot be invoked to explain the superiority of the 1 in

10 emulsion of the cholesterin-heart antigen over antigen 29 or the syphilitic liver extract, for the anticomplementary dose of these two extracts was only twice the amount used in the tests, while the 1 in 10 emulsion of the cholesterin-heart antigen was one fourth of the anticomplementary amount. The superiority of the cholesterin-heart antigens must be due to the peculiar lipoids extracted from the heart muscle. The ease in securing human hearts, and the comparatively cheap method of extracting a lipoid of constant value renders these extracts peculiarly suitable. The addition of cholesterin in suitable quantities increases the antigenic value of these extracts so that they are superior to any other antigen that we have tried.

SUMMARY AND CONCLUSIONS.

1. The addition of cholesterin to an alcoholic extract of heart or fetal liver increases the antigenic value of the extracts in the Wassermann reaction.
2. The optimum amount of cholesterin to be added to heart extract or fetal liver extract was found to be 0.4 per cent.
3. Cholesterin-heart extracts are superior to cholesterin-liver extracts and to alcoholic extracts of syphilitic livers, as well as to ether extracts of dried hearts.
4. Cholesterin-heart extracts prepared from different human hearts are practically equal in anticomplementary and antigenic value. Similar extracts prepared from guinea pig hearts have the same antigenic value as those prepared from human hearts. Both the human heart and the guinea pig heart extracts are superior to beef heart extract when the same amount of cholesterin is added to each of the extracts.
5. In testing blood serum for diagnostic purposes, it is not safe to use more than one fourth of the anticomplementary dose of the 0.4 per cent. cholesterin heart extract. In the work here presented, this consisted of a 1 in 10 emulsion.
6. In testing cerebrospinal fluids, 1 in 10 emulsions give slightly better reactions with smaller quantities of the fluid than do 1 in 6 emulsions.
7. Because of the simple preparation, the superior antigenic prop-

erty, and the constant antigen value of cholesterin-heart extracts prepared from human hearts, we agree with McIntosh and Fildes that this form of extract fulfills the requirements of a standard antigen.

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THE CEREBROSPINAL FLUID IN SYPHILIS.*

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The results presented in this communication are based on the examination of 113 cases of syphilis in all stages of the disease, from the secondary eruption to general paresis. During the past ten months every case of syphilis admitted to the hospital has had lumbar puncture performed as part of the routine examination; previous to that time only cases which suggested infection of the nervous system were punctured.

TECHNIQUE.

Lumbar puncture is always performed with the patient lying on the right side. It has been found that a uniform position for all patients greatly increases the ease of the procedure, and special stress is laid on having the patient properly placed in the beginning. The troublesome headache sometimes seen after lumbar puncture is less frequent with the patient prone, than when the puncture is performed with the patient sitting up. The skin and underlying tissues at the site of puncture are infiltrated with a little 2 per cent. novocaine solution. This makes the procedure much less painful and patients rarely object to repeated punctures. The routine examination of the fluid is confined to four procedures,—estimation of pressure, cell count, globulin determination, and Wassermann reaction.

Pressure.—The pressure is estimated by allowing the fluid to run into a graduated manometer tube, with a bore three millimeters in diameter, and reading the height to which the fluid rises. This gives a figure which is, of course, relative only, but the normal variations

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in pressure are so large,—90 to 200 millimeters,—that a more accurate measure is unnecessary and only causes delay.

Cell Count.—The preparations should be made as soon as the fluid is removed, as the cells settle rapidly and adhere to the sides and bottom of the tube if the fluid is left standing; they may be counted at leisure, as long as sufficient time does not elapse for evaporation to occur. The counts are made with the pipette ordinarily used for leucocyte counts and the Thoma-Zeiss counting chamber. Two pipettes and two chambers are used in each estimation. For diluting fluid we use 10 per cent. acetic acid in distilled water. This is drawn to the “1” mark on the pipette, which is then filled to the “11” mark with spinal fluid. This gives a dilution of spinal fluid of nine in ten within the chamber. The fluid in the capillary portion of the tube is then blown out, and a drop of fluid from the chamber run on to the counting stage. The cover-slip is then placed on the slide and, while firm pressure is being applied, pushed across the stage. With this method Newton rings are easily obtained on all four sides, thus assuring the constant depth of the preparation. The cells on the whole ruled surface are then counted. This ruled surface in the Thoma-Zeiss chamber has an area of 9 square millimeters. As the chamber is only $1/10$ of a millimeter in depth, the total count gives the number of cells in $9/10$ of a cubic millimeter of the diluted fluid. The figure so obtained must, therefore, be multiplied by $10/9$ to give the number of cells per cubic millimeter, and a further multiplication by $10/9$ is required to correct the dilution; or, combining the two calculations, we can multiply by $100/81$, that is approximately $10/8$. The result gives the number of cells in 1 cubic millimeter of undiluted fluid. The two chambers should correspond closely. This method has proved entirely satisfactory, and accurate within 10 per cent. In considering the findings the standard of Dreyfus (1), based on 750 punctures, is used:

1 to 5 cells per c. mm.....	normal.
6 to 9 cells per c. mm.....	doubtful, border cases.
10 to 20 cells per c. mm.....	slight lymphocytosis.
21 to 50 cells per c. mm.....	moderate.
Over 50 cells per c. mm.....	marked.

Globulin.—We have used the butyric acid method of Noguchi (2), which has been found entirely satisfactory. In some cases the colloidal gold method of Lange was also used, but in our hands this proved no more delicate and was less convincing than the Noguchi method. We have compared the Nonne-Apelt phase I reaction with the Noguchi butyric acid reaction in a number of fluids and have found the latter much the more delicate of the two. Where there is a marked excess of globulin, the results of the two reactions are quite parallel, but when the excess is slight, the phase I reaction fails, and fluids giving the degree of reaction classed by us as a $\pm$ reaction, that is, a faint haze with butyric acid, are with the Nonne-Apelt reaction entirely negative. The reagents used in the Noguchi reaction are butyric acid (Merck) diluted to 10 per cent. in 0.85 per cent. sodium chloride solution and a 4 per cent. solution of sodium hydrate. To 0.2 of a cubic centimeter of spinal fluid, 0.5 of a cubic centimeter of butyric acid is added and the mixture brought to a boil over a small flame; 0.1 of a cubic centimeter of 4 per cent. sodium hydrate is then added and the whole again heated to boiling. If carefully observed, the reaction can be delicately shaded and relatively quantitative readings made. The tubes should be watched carefully for the first ten minutes and read in half an hour and again in two hours. When possible several fluids should be done at the same time to obtain a comparison. In reading the Noguchi reaction we have adopted the following arbitrary standard:

- opalescence to very faint haze.
- $\pm$ faint haze to haze.
- +.....fine granular precipitate.
- ++.....heavy granular or coarse flocculent precipitate.
- +++.....very heavy flocculent precipitate.

Wassermann Reaction.—The introduction by Hauptmann and Hössli (3) in 1910 of the use of large quantities of spinal fluid in the Wassermann reaction entirely altered previous conceptions as to the value of this test in the examination of the spinal fluid. Previous to this it had been shown that of the four reactions used in determining the syphilitic nature of a disease of the nervous system,—Wassermann reaction in the blood, pleocytosis, globulin reac-

tion, and Wassermann reaction in the spinal fluid,—the latter was much the least frequently present. This was unfortunate as, when present, it was the most convincing of the four. The introduction of large amounts, five times as much fluid being used as is used in performing the reaction with serum, has made a positive Wassermann reaction in the spinal fluid one of the most constant findings in syphilis of the central nervous system. The test has, moreover, the great advantage that as yet it has never been found positive except where actual syphilitic infection of the central nervous system may logically be considered to have been present. Fraenkel (4) has checked by autopsy or operation the findings with these large amounts of fluid in thirty-six cases. In no case was a positive reaction obtained where syphilis of the central nervous system was not present.

In doing the Wassermann reaction we have used the anti-sheep hemolytic system with guinea pig complement. Daily titrations of complement have been made and the guinea pig serum has been diluted so that 0.5 of a cubic centimeter, the amount used in the test, contained two hemolytic units. Two units of hemolytic amboceptor have also been employed. The antigen used in most of the work was an alcoholic extract of human fetal liver, to which 0.25 per cent. cholesterin had been added. Latterly we have used an alcoholic extract of human heart to which 0.4 per cent. cholesterin had been added. The latter, which is a much more powerful antigen, was used with only a few of the fluids in this series. The findings in blood and spinal fluid are comparable, as reactions were always done with the same reagents. All the reagents were used in half the amount described in the original Wassermann reaction, giving a total volume of 2.5 cubic centimeters instead of the 5 cubic centimeters originally described. In some of the earlier fluids, only 0.1 of a cubic centimeter of fluid was used, but the majority were tested with 0.5, 0.4, 0.3, 0.2, 0.1, and 0.05 of a cubic centimeter (tables I to IX).¹ Twice the largest amount used in the test, that is, 1 cubic centimeter, was always used as anticomplementary control.

¹ As only half quantities of all reagents were used in performing the reaction, these amounts correspond to double the amount, or the 1.0 c.c., 0.8 c.c., 0.6 c.c., 0.4 c.c., etc., of Hauptmann, Nonne, Stühmer, Fraenkel, and others.

In no instance has the fluid been found to be anticomplementary in this amount, except in one purulent fluid from a case of epidemic meningitis. The following standard was adopted in reading the reaction:

- ++.....no hemolysis.
- +±.....slight tingeing of supernatant fluid.
- +.....50 per cent. of cells not hemolyzed.
- ±.....few cells not hemolyzed.
- complete hemolysis.

An attempt has been made to classify into groups, according to the clinical and pathological data obtainable, the patients whose spinal fluid findings are here reported. In some cases this has been easy and certain, but necessarily in a disease so complex and manifold as syphilis of the central nervous system, this grouping must in many cases be regarded as arbitrary. The following classification has been adopted: (1) secondary syphilis untreated; (2) secondary syphilis treated; (3) secondary syphilitic meningitis (including nerve relapses); (4) latent tertiary syphilis; (5) tertiary syphilitic meningitis; (6) syphilitic spinal spastic paralysis; (7) cerebrospinal syphilis; (8) cerebral syphilitic endarteritis; (9) tabes dorsalis; (10) general paresis.

SECONDARY SYPHILIS UNTREATED.

In 1903 Ravaut (5) reported on the examination of the spinal fluid in 116 cases of secondary syphilis. He found some degree of abnormality, either increased cells or increased protein content, in 67 per cent. Little attention was paid to these findings until the occurrence of the so called nerve relapses following treatment with salvarsan aroused fresh interest in the subject. In the past year several observers have examined the spinal fluid in cases of early secondary syphilis. All report frequent abnormal findings, and some give very high figures. Dreyfus (6) examined twenty-two cases of secondary syphilis and found changes in the spinal fluid in all, increased cells or protein in seventeen, and increased pressure in five. In a later paper (7) he states that 80 per cent. of cases of secondary syphilis show abnormalities in the spinal fluid. Wechselmann (8), basing his opinion chiefly on the results of the colloidal gold reaction of Lange, states that the majority of cases of syphilis show increased protein content of the spinal fluid. Boas and Lind (9) examined the spinal fluid in five cases of secondary syphilis and found counts of 9, 5, 3, 0, and 9. The Nonne-Apelt reaction was positive in two of the fluids; one of these had nine, the other no cells. The Wassermann reaction in the spinal fluid was negative in all (double amount used). Fraenkel (10) examined fifteen cases of early untreated

secondary syphilis. The fluid from five of these patients showed a definite lymphocytosis, in one case marked (272 cells per c. mm.); four other fluids showed counts between six and ten. The phase I reaction was positive in only one fluid. Five of the fifteen fluids gave a positive Wassermann reaction.

TABLE I.
Secondary Syphilis Untreated.

Name and case No.		Blood.	Cerebrospinal fluid.								
		Wassermann reaction.	Pressure in mm.	Cell count per c.mm.	Noguch globulin reaction.	Wassermann reaction in spinal fluid.					
						0.5 c.c. (1.0 c.c.) ²	0.4 c.c. (0.8 c.c.)	0.3 c.c. (0.6 c.c.)	0.2 c.c. (0.4 c.c.)	0.1 c.c. (0.2 c.c.)	0.05 c.c. (0.1 c.c.)
J. L.	658	++	170	0	—	—	—	—	—	—	—
J. K.	636	++	140	1	—	—	—	—	—	—	—
G. S.	647	++	200	1	—	—	—	—	—	—	—
G. F. J.	717	++	175	1	—	—	—	—	—	—	—
J. O'D.	874	++	120	1	—	—	—	—	—	—	—
J. B. W.	741	++	170	2	—	—	—	—	—	—	—
L. M.	656	++	140	2	—	—	—	—	—	—	—
W. S.	649	++	170	2	—	—	—	—	—	—	—
P. C.	700	++	170	5	—	—	—	—	—	—	—
E. W.	882	++	180	2	—	—	—	—	—	—	—
W. B. M.	526	++	100	2	—	—	—	—	—	—	—
L. G.	885	++	85	4	—	—	—	—	—	—	—
J. C.	657	++		5	—	—	—	—	—	—	—
O. H.	683	++	200	5	—	—	—	—	—	—	—
M. F.	780	++		2	±	—	—	—	—	—	—
G. B.	644	++	170	1	±	—	—	—	—	—	—
H. R.	714	+	180	1	±	—	—	—	—	—	—
N. R.	665	+	120	7	±	—	—	—	—	—	—
J. H.	661	++		8	±	—	—	—	—	—	—
C. J.	884	+ ±	160	12	—	—	—	—	—	—	—
T. R.	157	++	180	12	±	—	—	—	—	—	—
L. R.		+ ±	270	13	±	—	—	—	—	—	—

We have examined the spinal fluid of twenty-two cases of early untreated secondary syphilis. Three showed cell counts of 12, 12, and 13, respectively; that is, slight but definite lymphocytosis. Two of these had also slight increase of globulin. Two with counts of seven and eight belong to the doubtful, border-line group; both of these had also, however, some increase of the globulin. Three cases with normal cell counts showed increased globulin. In only one case could the pressure be considered abnormal (270 millimeters). The Wassermann reaction was in every case positive in the blood and negative in the spinal fluid. Eight patients of

² See footnote 1, page 474.

the twenty-two examined, or 36 per cent., showed some abnormality of the spinal fluid. Of these eight patients, three have been lost track of, two have shown a normal spinal fluid after intensive treatment with salvarsan and mercury, one (case 157) has attended very irregularly and has had only three intravenous injections of salvarsan; his fluid six months later and again eighteen months later was entirely negative. One (case 644), who had increased globulin but no increase in cells has, in spite of two courses of neosalvarsan and one course of mercury injections, shown the same findings on both of two subsequent examinations, the globulin reaction being indeed more marked at the last examination. Another patient (L. R.) has, in spite of twelve intravenous injections of neosalvarsan and two courses of mercury injections, developed a definite meningitis with double optic neuritis; this is now, however, yielding to further treatment.

SECONDARY SYPHILIS TREATED.

All the patients in this group had been previously treated with salvarsan. These cases are patients who had received their treatment before routine lumbar punctures were made. With the exception of case 193, none of the patients showed symptoms referable to the nervous system. Case 193 was admitted in June, 1911, with active secondary syphilis and was given four intravenous injections of salvarsan, totaling 0.9 of a gram. The Wassermann reaction became negative in August, 1911, but was again positive in October, 1911. During November she had five intravenous injections of 0.2 of a gram of salvarsan. In January, 1912, she was admitted with a severe facial neuralgia of three weeks' duration. Physical examination showed nothing abnormal. Lumbar puncture yielded a spinal fluid with a cell count of 14; the globulin was negative. She was given 0.3 of a gram of salvarsan and the pain entirely disappeared within twelve hours and has not returned. She has since had 1.5 grams of salvarsan and 5.1 grams of neosalvarsan. Subsequent lumbar punctures showed on May 28, 1912, eleven cells, and on December 7, 1912, seven cells; there has never been any increase in the globulin. Table II shows that of ten cases of

TABLE II.
Secondary Syphilis Treated.

Name and case No.	Blood.	Cerebrospinal fluid.									
		Wasser- mann reaction.	Pres- sure in mm.	Cell count per c.mm.	Nogu- chi glob- ulin re- action.	Wassermann reaction.					
						0.5 c.c. (1.0 c.c.) ³	0.4 c.c. (0.6 c.c.)	0.3 c.c. (0.6 c.c.)	0.2 c.c. (0.4 c.c.)	0.1 c.c. (0.2 c.c.)	0.5 c.c. (0.1 c.c.)
P. M. 433	+	±	80	6	—	—	—	—	—	—	—
H. H. C. 37	—	—		4	—	—	—	—	—	—	—
A. W. C. 13	—	—		4	—	—	—	—	—	—	—
W. S. F. 39	—	—	120	3	—	—	—	—	—	—	—
A. F. 71	—	—	170	5	—	—	—	—	—	—	—
R. M. H. 18	—	—	170	5	—	—	—	—	—	—	—
R. H. 586	±	—	160	3	—	—	—	—	—	—	—
J. C. 613	++	—	160	6	—	—	—	—	—	—	—
C. S. D. 193	—	—		14	—	—	—	—	—	—	—
H. G. 73	++	—	180	1	—	—	—	—	—	—	—

Tertiary Syphilis Treated.

J. H. T. 167	+	—	140	1	—	—	—	—	—	—	—
J. P. H. 642	++	—	110	0	—	—	—	—	—	—	—
F. L. 70	±	—	140	1	—	—	—	—	—	—	—
B. S. 204	+	—	140	2	—	—	—	—	—	—	—
A. D. 1,093	++	—		4	—	—	—	—	—	—	—
J. Z. 654	++	—	70	1	+	—	—	—	—	—	—

treated secondary syphilis, one, or 9 per cent., showed an abnormal fluid. This patient showed also clinical evidence suggesting involvement of the nervous system. Two showed doubtful or border-line counts with no increase of globulin.

SECONDARY SYPHILITIC MENINGITIS.

This group is made up of patients who had an evident meningitic involvement in the active secondary period; that is, in the first year of the disease. They should, perhaps, be included in class 2 (secondary syphilis treated), as all had had more or less salvarsan. Only four of these patients, however, belong in the group of patients with secondary syphilis treated by us and still under observation. The other four patients were admitted because involvement of the central nervous system was suspected. Moreover, none were discovered in the course of routine lumbar puncture, but all were examined because a lesion of the central nervous system was suspected. For these reasons they were classed separately.

³ See footnote 1, page 474.

TABLE III.
Secondary Syphilitic Meningitis.

Name and case No.	Wassermann reaction in blood.	Cerebrospinal fluid.						Time since chancre.	Time since first nervous symptom.	Treatment before first nervous symptom.	Treatment before lumbar puncture.				
		Pressure in mm.	Cell count per c.mm.	Noguchi globulin reaction.	Wassermann reaction.						Salvarsan.	Mercury.			
					0.5 c.c. (1.0 c.c.). ⁴	0.4 c.c. (0.8 c.c.)	0.3 c.c. (0.6 c.c.)						0.2 c.c. (0.4 c.c.)	0.1 c.c. (0.2 c.c.)	0.05 c.c. (0.1 c.c.)
D. M. T. 187	—		575	++	±	—	—	—	6 mos.	3 mos.	Salvarsan 0.7 gm. Mercury 4 injections	0.7 gm.	8 inj.		
P. H. 144	—		132	+	(±) ⁵	(—)	(—)	—	9 mos.	7½ mos.	Mercury per os 2 weeks	1.8 gm.	4 inj.		
M. H. 404	—	135	110	±	++	+	±	—	8 mos.	(?)	None	2.8 gm.	4 inj.		
A. B. R. 29	—	230	215	±	++	+	±	—	18 mos.	17 mos.	None	5.4 gm.	33 inj.		
C. W. W. 392	++	225	1074	++	(++)	(++)	(++)	++	5 mos.	1 mo.	Salvarsan 1.5 gm. Mercury 12 injections	1.5 gm.	12 inj.		
W. E. 655	+	190	190	±	++	+	±	—	6 mos.	5 mos.	None	1.0 gm.	Mercury per os 4 mos.		
F. S. 875	+	200	181	++	++	+	+	—	11 mos.	10½ mos.	None	0.5 gm.	8 inj.		
F. W. J. 730	+	210	30	+	—	—	—	—	7 mos.	6½ mos.	None	0.5 gm.	8 inj.		

⁴ See footnote 1, page 474.⁵ The readings in brackets indicate that at the time of the first examination of the fluid only 0.1 c.c. was used, the bracketed readings having been obtained on subsequent examination after the introduction of the use of larger quantities.

These cases include four typical examples of the so called neuro-relapse with paralysis of cranial nerves (cases 187, 392, 655, and 875). The clinical picture in these patients with secondary syphilitic meningitis varies greatly. In a previous paper (11) the relative insignificance of the clinical symptoms and signs which may be present in patients yielding such fluids has been pointed out. They may, on the other hand, show most extreme evidence of their nervous involvement. As will be seen by reference to table III, the spinal fluids of these patients show very consistent findings, more so than any other type of syphilis of the nervous system. Case 730 alone is irregular. We find, almost without exception, high cell counts, this condition yielding, as a rule, the most marked pleocytosis of any syphilitic condition of the nervous system. In our cases the globulin varied from a heavy flocculent precipitate ($++$ reaction) to a faint haze ($\pm$ reaction), but was positive in every case. Both the globulin and the number of cells paralleled closely the clinical condition, being most increased in the severe cases. The Wassermann reaction in the spinal fluid was positive with 0.5 of a cubic centimeter of fluid in every case but one, while with 0.1 of a cubic centimeter it was positive in only one. The Wassermann reaction in the blood was positive in four and negative in four cases, being apparently determined by the amount of previous treatment. These patients have responded satisfactorily to treatment, and all those who have been treated and followed (six) are now free from symptoms and all but one have normal spinal fluids. They are being kept under constant treatment with mercury and salvarsan in spite of the negative findings.

It is interesting to compare the findings in our cases of treated secondary syphilis with those of Altmann and Dreyfus (12), who examined the spinal fluid in eight cases of primary and fifty-six cases of untreated secondary syphilis. Two of the eight fluids from their primary cases showed cell counts of eight and thirteen respectively; both of these gave a positive phase I reaction. Seven of the fluids from the fifty-six patients with secondary syphilis, examined by them, had cell counts of between 75 and 500 cells per cubic millimeter; all the seven fluids showed also a marked increase

in the globulin and a positive Wassermann reaction. The Wassermann reaction in the spinal fluid was positive in eleven of the fifty-six cases; only once was it positive without an accompanying marked increase in cells and globulin. Many of the other fluids showed slighter changes, 66 per cent. in all of the fluids from fifty-six untreated secondary cases showing some increase in cells or globulin.

As will be seen by reference to table I, the number of our patients with untreated secondary syphilis who showed abnormalities in the spinal fluid is much smaller than that found by Altmann and Dreyfus, and the changes are much less marked. The discrepancies may be due to the fact that nearly all the patients were in the very early secondary stage. Altmann and Dreyfus found the marked changes,—cell counts of over 100, strong globulin reaction, and positive Wassermann reaction in the spinal fluid,—only in the later secondary cases. Table III shows that at this period of the disease we also had a number of patients whose spinal fluids showed similar findings. If the question of previous treatment with salvarsan be omitted, our findings in forty cases of secondary syphilis, cell counts of 30 to 1,074 in eight, positive Wassermann reaction in the spinal fluid in seven, and slight increase in cells or globulin in eleven, are comparable to those of Altmann and Dreyfus. From these figures it would appear, contrary to what is commonly held, that changes in the spinal fluid are not more frequent in treated than in untreated patients. These findings, meaning, as they almost certainly do, an infection of the central nervous system with *Spirochæta pallida*, emphasize the frequency of involvement of the central nervous system in syphilis and how early in the disease this invasion occurs.

LATENT TERTIARY SYPHILIS (WITHOUT NERVOUS MANIFESTATIONS).

Six patients were examined (table II, part 2) and in only one was any abnormality in the spinal fluid detected. This patient was admitted with lobar pneumonia. She denied syphilis, but gave a history of repeated miscarriages. The Wassermann reaction in the blood was strongly positive. Her spinal fluid showed no increase in cells, but gave a well marked globulin reaction.

TABLE IV.
Tertiary Syphilitic Spinal Meningitis.

Name and case No.	Blood	Cerebrospinal fluid.								
	Wassermann reaction.	Pres- sure in mm.	Cell count per c. mm.	Nogu- chi glob- ulin re- action.	Wassermann reaction.					
					0.5 C.C. (1.0 C.C.) ⁶	0.4 C.C. (0.8 C.C.)	0.3 C.C. (0.6 C.C.)	0.2 C.C. (0.4 C.C.)	0.1 C.C. (0.2 C.C.)	0.5 C.C. (0.1 C.C.)
V. H. 565	+ ±	220	124	+++	++	++	++	++	++	±
I. P. 660	+	195	15	—	—	—	—	—	—	—
F. Z. 691	—	170	24	+	—	—	—	—	—	—

TERTIARY SYPHILITIC MENINGITIS.

In this group (table IV) were placed those patients whose symptoms and signs could be explained by inflammatory thickened meninges pressing on nerve roots, and in whom no evidence of lesions of the actual nervous tissue of the cord could be obtained. The findings in the spinal fluid in the three cases were varied.

TABLE V.
Syphilitic Spinal Spastic Paralysis.

Name and case No.	Blood.	Cerebrospinal fluid.								
	Wassermann reaction.	Pressure in mm.	Cell count per c.mm.	Noguchi globulin reaction.	Wassermann reaction.					
					0.5 c.c. (1.0 c.c.) ⁷	0.4 c.c. (0.8 c.c.)	0.3 c.c. (0.6 c.c.)	0.2 c.c. (0.4 c.c.)	0.1 c.c. (0.2 c.c.)	0.05 c.c. (0.1 c.c.)
R. W. 94	±		5	++						
G. L. 603	++	260	36	++	++	++	++	++	±	—
J. McE. 342	++	165	10	++					—	
B. F. W. 933	+ ±	120	20	+	—	—	—	—	—	—

SYPHILITIC SPINAL SPASTIC PARALYSIS.

Case 603 (table V) is a typical example of the type of spastic paraplegia first differentiated and described by Erb, and especially studied by Oppenheim and by Nonne. The condition consists of a slowly developing spastic paralysis of the lower extremities, with spastic gait, muscular weakness, increased tendon reflexes, and

⁶ See footnote 1, page 474.⁷ See footnote 1, page 474.

bladder disturbance. The upper extremities are normal and there is no change in mentality, cranial nerves, or pupils, and no disturbance of sensation. The other three patients present almost an identical clinical picture, but in them the onset was in every case sudden. In these cases the lesions are usually considered to be chiefly vascular, and the findings in the spinal fluid,—low cell count and strong globulin reaction,—support this view.

CEREBROSPINAL SYPHILIS.

There is probably no pathological condition the signs and symptoms of which are so manifold and varied as cerebrospinal syphilis. This is to be expected from the variation in site and character which the process exhibits. The cerebrospinal fluid, on the other hand, shows a fairly constant picture (table VI), the greatest variation being in the magnitude of the cell increase. Almost every case shows a strong globulin reaction and a positive Wassermann reaction in the spinal fluid. The difference in the number of positive Wassermann reactions in the spinal fluid when 0.5 of a cubic centimeter is used, instead of 0.1 of a cubic centimeter, is well illustrated in this condition. With 0.1 of a cubic centimeter, only seven cases out of twenty-one, or 33 per cent., were positive, while with 0.5 of a cubic centimeter, fifteen out of sixteen fluids examined, or 94 per cent., gave a positive reaction. Hauptmann (13) examined forty-four cases of cerebrospinal syphilis which were negative with the smaller amount of fluid; of these forty-two were positive when larger amounts of fluid were used. One or two of the cases, whose spinal fluid findings are given in table VI, will be given in more detail, as they illustrate the slight signs and symptoms which may be shown by patients whose spinal fluid shows them to be victims of extensive active disease. Case 913 had complete loss of reaction to both light and accommodation in both pupils. This was of eleven years' duration. He had no symptoms beyond disturbed vision, and except for the pupil change and the absence of one tendon Achilles reflex, his physical examination was negative. Case 605 had a similar paralysis of accommodation in one eye of two years' duration; he has had no symptoms

TABLE VI.
Cerebrospinal Syphilis.

Name and case No.	Blood.	Cerebrospinal fluid.										Years since chancre.	Secondaries.	Time since onset of nervous symptoms.					
		Wassermann reaction.	Pressure in mm.	Cell count per c.mm.	Noguch's globulin reaction.	Wassermann reaction.					0.5 c.c. (1.0 c.c.) ^a				0.4 c.c. (0.8 c.c.)	0.3 c.c. (0.6 c.c.)	0.2 c.c. (0.4 c.c.)	0.1 c.c. (0.2 c.c.)	0.5 c.c. (0.1 c.c.)
						0.5 c.c. (1.0 c.c.) ^a	0.4 c.c. (0.8 c.c.)	0.3 c.c. (0.6 c.c.)	0.2 c.c. (0.4 c.c.)	0.1 c.c. (0.2 c.c.)									
M. C. 913	++	210	13	++	++	++	++	++	++	++	++	++	++	++	++	++	20	Slight	11 yrs.
F. K. 605	++	160	60	++	++	++	++	++	++	++	++	++	++	++	++	++	Denied	2 yrs. (?)	
H. G. 1,199	-	100	58	++	++	++	++	++	++	++	++	++	++	++	++	++	Denied	3 yrs. (?)	
J. H. R. 154	++	63	63	++	++	++	++	++	++	++	++	++	++	++	++	++	Denied	2 yrs.	
L. G. 888	+	150	53	++	++	++	++	++	++	++	++	++	++	++	++	++	11	Moderate	9 mos.
W. S. 1,118	++	230	183	++	++	++	++	++	++	++	++	++	++	++	++	++	6½	None	6 yrs. (?)
M. P. 794	-	120	40	+	(++) ^b	(++)	(++)	(++)	(++)	(++)	(++)	(++)	(++)	(++)	(++)	(++)	3½	Moderate	1 yr.
J. R. W. 887	±	135	135	++	++	++	++	++	++	++	++	++	++	++	++	++	15	Moderate	1½ yrs.
H. B. 934	+	240	233	++	++	+	-	-	-	-	-	-	-	-	-	-	13	Moderate	2 yrs.
D. J. W. 806	-	260	98	++	++	++	++	++	++	++	++	++	++	++	++	++	4	Moderate	4 mos.
R. B. 1,201	-	230	22	++	++	++	++	++	++	++	++	++	++	++	++	++	5½	Moderate	1 yr.
L. C. 562	-	260	65	++	++	++	++	++	++	++	++	++	++	++	++	++	2	Moderate	1 wk.
A. DeL. 153	-	70	35	++	++	++	++	++	++	++	++	++	++	++	++	++	17	(?)	13 yrs.
V. der H. 52	++	220	5	++	++	++	++	++	++	++	++	++	++	++	++	++	7	Moderate	4 mos.
R. O. 168	++	165	2	+	++	++	++	++	++	++	++	++	++	++	++	++	Denied	1 yr. (?)	
F. B. 345	++	140	10	++	++	++	++	++	++	++	++	++	++	++	++	++	20 mos.	Moderate	7 mos.
U. C. B. 1,032	±	140	50	++	++	++	++	++	++	++	++	++	++	++	++	++	Denied	1 yr. (?)	
L. G. 720	++	110	90	++	++	++	++	++	++	++	++	++	++	++	++	++	Congenital	1 wk.	
C. G. 1,304	-	100	38	++	++	++	++	++	++	++	++	++	++	++	++	++	25	Moderate	3 yrs.
J. M. 593	++	100	38	++	++	++	++	++	++	++	++	++	++	++	++	++	7	Moderate	2 yrs.
L. H. 937	-	230	5	±	-	-	-	-	-	-	-	-	-	-	-	-	31	Moderate	18 yrs.
Positive, per cent.	62	86	100	94	88	88	81	33	25										

Total No. of cases 21.

^a See footnote 1, page 474.^a The readings in brackets indicate that at the time of the first examination of the fluid only 0.1 c.c. was used, the bracketed readings having been obtained on subsequent examination after the introduction of the use of larger quantities.

beyond two momentary attacks of slight dizziness, and his physical examination was otherwise entirely negative. Cases 154 and 1,199 are similar cases of isolated pupil disturbance; they have, now, however, developed other signs of nervous involvement. Case 888 had no symptoms except "general nervousness" and some chronic gastric distress; his physical examination was entirely negative. Case 887 had headaches of eighteen months' duration and deafness in one ear; his physical examination showed beginning optic atrophy and nerve deafness in the affected ear; he had visited numerous eye and ear dispensaries, but had been given no antisyphilitic treatment; his headache disappeared within forty-eight hours after starting treatment. Case 1,032, a man aged thirty-eight years, noticed in December, 1911, some unsteadiness of station. In February, 1912, he was taken with sudden weakness of the lower extremities which gradually increased until June, 1912, when he became confined to bed. He entered the hospital on November 29, 1912, with a complete flaccid paralysis of the lower extremities, weakness of the muscles of the back, tingling of the fingers, and some weakness and huskiness of voice. He showed slightly unequal contracted pupils which reacted sluggishly to light and accommodation, areas of anesthesia to light touch over the trunk and lower extremities, and a complete flaccid paralysis of the lower extremities, with loss of all tendon reflexes. There was slight atrophy of both lower extremities with marked muscular hypotonus, fibrillary twitching, and loss of myotatic irritability. The reaction of degeneration was not present. This is apparently an example of syphilitic anterior poliomyelitis. Nonne (14) has collected a number of such cases from the literature and reports three cases of his own. The sensory changes in this case, however, remove it from the pure cases of this type, in which evidence of lesions in the anterior horn alone is found. Case 720 is similar. This child was sent in as a case of acute anterior poliomyelitis; the true nature of the disease was only suspected when it continued to progress. Under specific treatment improvement rapidly occurred.

CEREBRAL SYPHILITIC ENDARTERITIS.

In this group (table VII) we have placed the cases whose clinical and spinal fluid findings suggest that their symptoms are

due to vascular disease rather than to a syphilitic infection of the actual nervous tissue. These patients have not, in all probability, a syphilitic infection of the central nervous system, any more than has a case of aortitis, the disturbance of the nervous system in them being entirely secondary and accidental, dependent only on impaired nutrition due to vascular disease. The clinical diagnosis has since

TABLE VII.

Cerebral Endarteritis (?). Indefinite Cerebral Symptoms. Negative Findings.

Name and case No.	Blood.	Cerebrospinal fluid.								
	Wassermann reaction.	Pressure in mm.	Cell count per c.mm.	Noguchi globulin reaction.	Wassermann reaction.					
					0.5 c.c. (x.0 c.c.) ¹⁰	0.4 c.c. (0.8 c.c.)	0.3 c.c. (0.6 c.c.)	0.2 c.c. (0.4 c.c.)	0.1 c.c. (0.2 c.c.)	0.05 c.c. (0.1 c.c.)
D. T. 568	—		1	+	—	—	—	—	—	—
M. L. S. 427	—	210	1	—	—	—	—	—	—	—
M. C. C. 662	—	170	2	—	—	—	—	—	—	—
W. S. H. 746	—	120	4	—	—	—	—	—	—	—
J. M. S. 422	++	140	2	—	—	—	—	—	—	—
B. T. 528	—	150	3	—	—	—	—	—	—	—

been confirmed in one case at autopsy. Nonne (15) has called attention to the absence or trivial character of the spinal fluid findings in this type of case. Clinically they showed indefinite cerebral symptoms,—headache, dizziness, “nervousness,”—in short, the ordinary symptoms of cerebral arteriosclerosis. Physical examination in these patients was negative, and the spinal fluid showed no abnormality, except, in one of our cases, an increase of globulin.

TABES DORSALIS.

The examination of the spinal fluid in this condition yields information of great value. By this procedure an early diagnosis can be confirmed, and a valuable index of the result and efficiency of subsequent therapy is obtained. Moreover, no adequate conception of the course of the syphilitic process can be reached until all available sources of information have been investigated in each case, and until the history, physical signs, and findings in the blood and spinal fluid have been analyzed and correlated.

¹⁰ See footnote 1, page 474.

TABLE VIII.

Tabes Dorsalis.

Name and case No.	Blood.	Cerebrospinal fluid.										Years since chancre.	Secondaries.	Years since onset of tabes.
		Pressure in mm.	Cell count per c. mm.	Noguchi reaction.	Wassermann reaction.					0.05 c.c. (0.1 c.c.)				
					0.5 c.c. (1.0 c.c.) ¹¹	0.4 c.c. (0.8 c.c.)	0.3 c.c. (0.6 c.c.)	0.2 c.c. (0.4 c.c.)	0.1 c.c. (0.2 c.c.)					
F. H. P. 1,097	++	160	225	++	++	++	++	++	++	++	8	Slight	1½	
J. J. McV. 1,136	++	120	143	++	++	++	++	++	++	++	18	Severe	7	
C. M. 485	++	220	130	++	++	++	++	++	++	++	19	Denied	1½	
C. L. V. 85	++	120	125	++	++	++	++	++	++	++	16	Denied	6	
A. T. 1,177	-	180	114	++	(+)	++	++	++	++	++	8	Slight	1	
O. K. 1,159	++	120	112	++	++	++	++	++	++	++	12	Slight	4	
O. W. A. 213	++	180	110	++	++	++	++	++	++	++	9	Moderate	2½	
C. M. A. 1,111	±	180	92	++	++	++	++	++	++	++	17(?)	Moderate	11	
W. L. S. 113	++	60	72	++	++	++	++	++	++	++	23	Moderate	7	
A. D. 1,140	++	180	57	++	++	++	++	++	++	++	14	Moderate	1½	
M. B. 517	-	200	55	+	±	++	±	-	-	-	Denied	Denied	3	
R. d'H. 674	++	130	55	+	++	++	++	++	++	++	Chan- croids 7	Denied	3	
S. C. 560	+	200	50	++	±	++	±	-	-	-	14	Denied	Tables optica 1	
R. S. P. 1,186	++	130	47	++	++	++	++	++	++	++	Denied	Denied	4 mos.	
O. J. L. 836	-	100	45	±	-	++	-	-	-	-	15	Marked	11	
A. N. P. 903	++	180	44	±	++	++	++	++	++	++	16	Very slight	10(?)	
H. S. R. 1,042	-	230	42	-	++	++	++	++	++	++	Denied	Denied	3	
C. H. L. 557	+	40	42	++	++	++	++	++	++	++	30	Denied	10	
F. B. 893	+	120	40	±	±	++	-	-	-	-	Chan- croids 13	Denied	2	
H. G. G. 675	-	160	35	±	++	++	++	++	++	++	Denied	Denied	2½	
J. A. B. 1,141	++	180	33	++	++	++	++	++	++	++	32	Denied	2	
J. J. S. 559	++	180	30	++	±	++	±	++	++	++	8	Denied	1½	
O. F. 152	++	75	27	++	++	++	++	++	++	++	10	Denied	3½	
W. P. K. 980	-	50	16	++	++	++	++	++	++	++	12	Denied	6	

The Cerebrospinal Fluid in Syphilis.

Name and case No.	Blood.		Cerebrospinal fluid.							Years since onset of tabes.			
	Wasser- mann reaction.	Pressure in mm.	Cell count per c. mm.	Neguchii reaction.	Wassermann reaction.						Years since chancre.	S con- daries.	
					0.5 c.c. (1.0 c.c.) ¹¹	0.4 c.c. (0.8 c.c.)	0.3 c.c. (0.6 c.c.)	0.2 c.c. (0.4 c.c.)	0.1 c.c. (0.2 c.c.)				0.05 c.c. (0.1 c.c.)
P. J. F.	—	130	14	+	—	—	—	—	—	—	8	Mild	1
C. L. B.	—	100	9	—	—	—	—	—	—	—	8(?)	Denied	4(?)
J. F. E.	±		9	+	++	—	—	—	—	—	23	Moderate	12
B. A.	—		8	±	—	—	—	—	—	—	29	Denied	13
B. F. Y.	±	160	7	+	—	—	—	—	—	—	15	(?)	3 to 7
J. W.	++	150	3	—	—	—	—	—	—	—	10	Denied	1½
Positive, per cent.	66		97 83	87	86	83	69	55	23	4			

Total number of cases 30.

¹¹ See footnote I, page 474.

¹² The readings in brackets indicate that at the time of the first examination of the fluid only 0.1 c.c. was used, the bracketed readings having been obtained on subsequent examination after the introduction of the use of larger quantities.

Thirty patients with *tabes dorsalis* have been under observation and treatment in the hospital. The spinal fluids of all but one of these patients showed an increase in cells. Four cases showed, however, cell counts between six and nine. These must be looked upon as border-line counts and cannot be regarded as true lymphocytosis. Two of these four patients (cases 847 and 848) had each had two injections of salvarsan at other hospitals before admission. If we omit these doubtful cases, twenty-five, or 83 per cent., of the fluids examined showed a true lymphocytosis. The globulin reaction was positive in twenty-six cases, or 87 per cent. The Wassermann reaction in the spinal fluid, with 0.1 of a cubic centimeter of the fluid, was positive in seven and negative in twenty-three, giving 23 per cent. of positive reactions; two of the seven positive reactions were only weakly positive. With 0.5 of a cubic centimeter of fluid the reaction was positive in twenty-four and negative in four, giving 86 per cent. of positive reactions. It will thus be seen that lymphocytosis, increased globulin, and a positive Wassermann reaction in the spinal fluid occur with practically equal frequency. One patient with an outspoken *tabes* (case 524) had a perfectly normal spinal fluid.

The Wassermann reaction in the blood is less frequently present than the other three reactions. In this series twenty patients, or 66 per cent., had a positive reaction in the blood. The results of the Wassermann reaction in this series of thirty patients (66 per cent. positive in the blood, 86 per cent. positive in the spinal fluid), are in striking agreement with those of Hauptmann (13), who, in a similar series of tabetics, found 66 per cent. positive in the blood, and 87 per cent. positive in the spinal fluid. One of us (Swift (16)) in a previous series of tabetics also found 66 per cent. of positive reactions in the blood.

A striking fact brought out in table VIII is the trifling character of the secondary manifestations of syphilis in the majority of patients subsequently developing *tabes dorsalis*. Sixteen of the thirty patients, or more than 50 per cent., denied all knowledge of secondary manifestations. This is a rule to which attention has been called by numerous observers, and has been especially emphasized by Mott (17). The figures are more striking if one

compares the findings in cerebrospinal syphilis (table VI), where only five of the twenty-one patients denied secondaries. Moreover, three of the five cases of cerebral syphilis denying secondaries (cases 605, 1,199, and 154) are patients with an ophthalmoplegia as practically the sole evidence of their disease,—a class of case which, Nonne (14) states, almost always develops later into either tabes or paresis.

GENERAL PARESIS.

Only three cases of general paresis have been observed. Two of these were very early cases (Nos. 42 and 883). All three showed a positive Wassermann reaction in the blood, moderate or slight pleocytosis, a strong globulin reaction, and an intensely strong posi-

TABLE IX.
General Paresis.

Name and case No	Blood.	Cerebrospinal fluid.								
	Wasser- mann re- action.	Pres- sure in mm.	Cell count per c. mm.	Nogu- chi glob- ulin re- action.	Wassermann reaction.					
					0.5 c.c. (1.0 c.c.) ¹⁸	0.4 c.c. (0.8 c.c.)	0.3 c.c. (0.5 c.c.)	0.2 c.c. (0.4 c.c.)	0.1 c.c. (0.2 c.c.)	0.05 c.c. (0.1 c.c.)
A. 42	+	145	16	+++	++	++	++	++	++	++
M. N. 883	++	90	46	++	++	++	++	++	++	++
H. L. P. 1,172	++		9	++	++	++	++	++	++	+

tive Wassermann reaction in the spinal fluid. In two cases the fluid gave complete fixation of complement with 0.006 of a cubic centimeter of spinal fluid. The occurrence of a positive Wassermann reaction with such small amounts of spinal fluid, 0.05 of a cubic centimeter and less, seems, in our experience and that of others, to be of value in attempting to differentiate cases of general paresis from the gummatous and vascular forms of cerebral syphilis. Nonne and Holzmann (18) are of the opinion that these findings are also significant in attempting to recognize cases of taboparesis. Our experience in a limited number of cases tends to confirm this opinion. Only one of our tabetics (case 1,097) gave a positive reaction with this amount of fluid, 0.05 of a cubic centimeter. This patient had a definite change in personality and mental deterioration, and is almost certainly a case of taboparesis.

¹⁸ See footnote 1, page 474.

In conclusion we should like to emphasize the value of the frequent use of lumbar puncture in the care and study of syphilis. The procedure is simple, and the information obtained of extreme value. In considering prognosis and the amount of treatment likely to be required, the examination of the spinal fluid for possible abnormalities in the secondary stage of the disease is of the utmost importance. The diagnosis of any syphilitic condition of the central nervous system is not complete without an examination of the spinal fluid, and in the treatment of these conditions repeated examinations of the fluid furnish the best guide to therapy. A negative Wassermann reaction in the blood is no evidence that the nervous system is not affected and every patient with increased cells, globulin, or a positive Wassermann reaction in the spinal fluid needs active antisyphilitic treatment. No patient who has been infected with syphilis can conscientiously be discharged as cured, until the spinal fluid has been examined and found normal, even though he remains free from signs and symptoms and has a persistently negative Wassermann reaction in the blood.

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SECTION XIII.

DERMATOLOGY AND SYPHILOGRAPHY.

INDEPENDENT PAPER.

THE COMBINED LOCAL AND GENERAL TREATMENT OF SYPHILIS OF THE CENTRAL NERVOUS SYSTEM.

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The recent clinical studies of syphilis of the central nervous system and the development in laboratory methods of diagnosis have made it possible to ascertain the presence of active syphilis of the brain or spinal cord with a fair degree of accuracy. From the standpoint of efficient therapy, the investigations have been less fruitful. It is the purpose of this communication to present the results of an attempt to solve the problem of the most efficient method of application of our curative agents.

The demonstration by Noguchi and Moore¹ of the *Treponema pallidum* in the brain of patients with general paralysis has definitely established the fact that there is active syphilis of the central nervous system in the so-called parasyphilitic nervous diseases. In addition, the changes in the cerebro-spinal fluid in general paralysis and tabes are qualitatively the same as in cerebro-spinal syphilis. The pleocytosis, with a predominance of mononuclear forms, and the increase in globulin indicate the presence of a chronic inflammatory process in the cerebro-spinal axis. The positive Wassermann reaction in the fluid indicates that the process is syphilitic in nature. These abnormal constituents usually disappear more rapidly under specific treatment in secondary syphilitic meningitis than they do in late cerebro-spinal syphilis, or in tabes or general paralysis. This is, no doubt, due to the fact that in late syphilis the spiro-

chætæ have established themselves in strongholds difficult to reach with our drugs. These strongholds are the regions of poor vascularization, in which the central nervous system is particularly rich. The *Treponema pallidum* has a special predilection for lymph spaces, and for practical purposes the perivascular lymphatics and the perineuronal spaces may be considered as lymph spaces which are not in direct communication with the general lymphatic system, but are part of the subarachnoid space.² In syphilitic meningo-arteritis, the most intense pathological changes occur in the region of the perivascular lymphatics. In paresis the perineuronal lymph spaces are often filled by the round cell infiltration. Normally the perineuronal spaces and perivascular lymphatics are filled with cerebro-spinal fluid which also fills the subarachnoid space. It has been well established that there is very little excretion of curative agents from the blood into the cerebro-spinal fluid, so that in these chronic syphilitic diseases of the central nervous system, most of the curative action of our drugs is exerted on the side of the blood-stream, and there is little if any application of the therapeutic material by the cerebro-spinal fluid, which is in more intimate relation with the diseased tissue. Flexner³ and his co-workers have shown the necessity of introducing the therapeutic sera directly into the subarachnoid space in the various forms of purulent meningitis. The same principle no doubt holds good, to a considerable degree, in all other forms of meningitis.

In dealing with syphilis of the central nervous system we are, however, more fortunate than is the case in purulent meningitis; for here the introduction of our therapeutic agents into the general circulation is of undoubted benefit. With mercury and iodides and with salvarsan intravenously, much can be accomplished, and in many patients all clinical signs and symptoms can be relieved. Efficient therapy, however, means more than the relief of symptoms; it involves also the eradication of all evidence of active disease. This is more difficult. The changes occurring in the cerebro-spinal fluid,—pleocytosis, excess of globulin and positive Wassermann reaction,—must, as previously stated, be looked upon as evidence of active syphilis of the central nervous system. No treatment can be considered adequate which does not result in the permanent disappearance of these abnormal constituents.

While rapid symptomatic improvement frequently follows the use of mercury and iodides, the relatively slight influence of these drugs on the abnormal constituents in the cerebro-spinal fluid suggests at once the probability of a subsequent relapse. In tabes the use of mercury is frequently without benefit. Salvarsan intravenously acts more rapidly than mercury, especially in reducing the degree of pleocytosis. To cause the excess of globulin to disappear and to change the Wassermann reaction from positive to negative is, however, much more difficult.

The idea of intensifying the treatment of syphilis of the central nervous system by the introduction of salvarsan directly into the cerebro-spinal fluid naturally suggests itself. To determine whether this might be safely done, we⁴ injected small amounts of the drug, diluted with 40 per cent. monkey serum, intraspinaly into monkeys. Subsequent examination of their cerebro-spinal fluid showed that the drug introduced in this form was too irritating to warrant its application to patients. Neosalvarsan injected intraspinaly into monkeys was less irritating, but when injected into patients produced symptoms too severe to warrant its continued use. Wechselmann⁵ reports upon the injection of neosalvarsan intraspinaly into two patients with general paralysis, and into two children with congenital lues, without injurious effect. Marinesco,⁶ however, reports unfavorable results in thirteen patients, each of whom received 5 mgms. of neosalvarsan in .4 c.c. solution intraspinaly. In ten of these patients severe unpleasant symptoms immediately appeared. Eight of them had permanent bladder disturbance. From this experience, Marinesco advises strongly against the intraspinal injection of neosalvarsan. We are in complete accord with his conclusions.

The blood serum of patients treated intravenously with salvarsan has been shown to have definite therapeutic value when injected subcutaneously into patients with congenital and secondary syphilis.^{7, 8, 9} We¹⁰ have been able to show that this serum exerts a spirochætidal action on the spirochætæ of relapsing fever. When the serum is allowed to act on the spirochætæ in vitro and subsequently these are injected into mice, they do not develop in the mice as do untreated spirochætæ or spirochætæ on which normal serum

has been allowed to act. Heating the serum to 56° C. for thirty minutes increases the spirochæticidal action. Gonder¹¹ reports a similar spirochæticidal action of the blood serum of salvarsan-treated rats and hens. He¹² has also found that the *Spirochæta gallinarum* in infected ticks is killed when the ticks are allowed to suck the blood of salvarsan-treated hens. We have also used the serum of syphilitic patients to make serum agar for the growth of *Treponema pallidum* after Noguchi's method. Cultures in media made with serum obtained before treatment grew practically as well as in that made with normal serum, while in media made with serum obtained an hour after intravenous injections of salvarsan the spirochætæ grew more slowly or not at all. In media made with serum which was obtained at longer intervals after treatment the growth approximated that in normal control tubes. These experiments indicate that the serum has the greatest inhibitory effect shortly after the injection of salvarsan.

All of the above mentioned experiments support the view that the serum of salvarsan-treated patients has a definite antispirochætal effect both in vitro and in vivo. These sera are ideal preparations for direct introduction into the subarachnoid space, and for the past two years we have been studying the results of the injection of such sera intraspinously into patients with syphilitic disease of the central nervous system. The early results were given in a previous communication.¹⁸

Marinesco¹⁴ states that he had treated patients with syphilis of the central nervous system with serum obtained from patients with secondary syphilis after treatment with salvarsan. There were some beneficial results, but the work was not continued. Robertson¹⁵ reports on the intraspinal treatment of patients with general paralysis. For this treatment he used the serum of patients with secondary syphilis, which was withdrawn on the third day after a salvarsan injection, and also the paretics' own serum, withdrawn one hour after the intravenous injection of salvarsan. Only a few treatments were given to each patient, but half of those treated showed some improvement.

The technique that we have employed is as follows: One hour after the intravenous injection of salvarsan, 40 c.c. of blood is with-

drawn directly into bottle-shaped centrifuge tubes and allowed to coagulate, after which it is centrifugalized. The following day 12 c.c. of serum is pipetted off and diluted with 18 c.c. of normal saline. This 40 per cent. serum* is then heated at 56° C. for half an hour. After lumbar puncture the cerebro-spinal fluid is withdrawn until the pressure is reduced to 30 mm. cerebro-spinal fluid pressure. The barrel of a 20 c.c. Luer syringe (which has a capacity of about 30 c.c.) is connected to the needle by means of a rubber tube 40 cm. long. The tubing is then allowed to fill with cerebro-spinal fluid, so that no air will be injected. The serum is then poured into the syringe and allowed to flow slowly into the subarachnoid space by means of gravity. At times it is necessary to insert the plunger of the syringe to inject the last 5 c.c. of fluid. It is important that the larger part of the serum should be injected by gravity, and if the rubber tubing is not more than 40 cm. long, the pressure cannot be higher than 400 mm. Usually the serum flows in easily under even a lower pressure. By the gravity method the danger of suddenly increasing the intraspinal pressure to the danger point, such as might occur with rapid injection with a syringe, is avoided. Frequently there is a certain amount of pain in the legs, commencing a few hours after the injection. The pain is more often noticed in tabetics than in patients with cerebro-spinal syphilis. It can usually be controlled by means of phenacetin and codeine, but occasionally morphine is required.

The therapeutic effect of this form of treatment is illustrated in the tables, where the treatment, Wassermann reaction in the blood and in the cerebro-spinal fluid, the cell count and globulin in the fluid of each patient are given. The cell counts were all made with the counting chamber method, two preparations being made of each fluid immediately after the fluid was obtained. To test the globulin, we have used the butyric acid method of Noguchi,¹⁶ and in reading the reactions have adopted the following standard:

- opalescence to very faint haze.
- ± faint haze to haze.
- + ... fine granular precipitate.
- ++ heavy granular to coarse flocculent precipitate.

*In patients who do not have reactions following the injection of 40 per cent. serum, the strength is at times increased to 50 or 60 per cent., or even stronger.

We have compared the Nonne-Apelt phase I reaction with the Noguchi butyric acid reaction in a large number of fluids and have found the Noguchi reaction much the more delicate of the two. Where there is a marked excess of globulin, the results of the two reactions are quite parallel, but when the excess is slight, the phase I reaction fails; the fluids giving the degree of reaction designated in the tables as $\pm$ are entirely negative with the Nonne-Apelt reaction. In some of the tables the Wassermann reaction in the cerebro-spinal fluid is indicated with two different antigens. Prior to January, 1913, a fetal liver extract was used as antigen. It was then found that an alcoholic extract of human heart, to which 0.4 per cent. cholesterin had been added, was a more sensitive reagent.¹⁷ In order not to confuse the results, it was considered wise to continue the use of the liver antigen in those cases where it had previously been used and to add the comparative reactions with the cholesterin heart antigen. Unless otherwise designated in the notes, after January, 1913, a completely negative reaction indicates that it was negative with the cholesterin-heart as well as with the liver antigen. In the cerebro-spinal fluid column the strength of the reaction in the fluid is indicated.* The quantitative titration of the strength of the reaction recommended by Hauptmann and Hössle¹⁸ has been used by us since November, 1911. Previous to that time only 0.2 c.c. (0.1 c.c.) of fluid was used. The variation in the strength of the reaction shown by the titration method furnishes a fair index of the effect of treatment. In this report we wish to lay more emphasis upon the improvement in the condition of the cerebro-spinal fluid than upon the clinical improvement, because it is entirely objective and probably gives us a better idea of the actual effect of our therapeutic measures.

Case 85.—C. L. V. Had a genital ulcer without secondary symptoms sixteen years before admission. For six years he had had lightning pain in the legs, ataxia for two years, abdominal girdle sensation for one year, arthropathy of

* In performing the reaction with the cerebro-spinal fluid, 0.5 c.c., 0.4 c.c., 0.3 c.c., 0.2 c.c., 0.1 c.c. and 0.05 c.c. of fluid are used. As we employ one-half quantities of all the reagents as compared with the amounts originally recommended for the Wassermann reaction, these quantities correspond to 1.0 c.c., 0.8 c.c., 0.6 c.c., 0.4 c.c., 0.2 c.c. and 0.1 c.c. respectively. The latter figures are given in the tables to make the readings uniform with the results of other workers.

TABLE I.

No. 85. C. L. V., Age 36. *Tabs Dorsalis*, 6 Years; *Syphilis*, 16 Years.
Result—Improved.

Date.	Blood.	Cerebro-spinal Fluid.			Treatment.	
	Wassermann Reaction.	Cells per c.mm.	Noguchi Globulin.	Wassermann Reaction.	Intravenous.	Intraspinal Serum.
1910						
Nov. 10 to 1911	++				16 injections HgCl ₂ intramuscularly	
Jan. 10	+					
16	±	125	++	0.2 c.c. —	Salvarsan 0.2 gm.	
25	+				" 0.3 "	
Feb. 8	+				" 0.36 "	
13		150	±	0.2 " —		
Feb. 15 to Aug. 20	±				Mixed treatment by mouth	
21	±	63	+	0.2 " ±(?)		
Aug. 26 to Sept. 22	±(?)				Salvarsan 5x0.2 gm.	
22		26	±	0.2 " —		
24					" 0.2 "	
Dec. 1*	±(?)	50	+	1.0 " ±	" 0.2 "	18 c.c. of 50%
11	±	20	+		" 0.2 "	30 " of 50 "
18	±	20	±	1.0 " ±	" 0.2 "	20 " of 40 "
1912						
Jan. 5	—	12	±	1.0 " +±	" 0.3 "	30 " of 40 "
12	—	4	—	1.0 " ±	" 0.3 "	30 " of 50 "
15	—				" 0.2 "	
20		5	—	1.0 " ±(?)		
July 9	—	2	—	1.0 " —		
Sept. 10	—	1	—	1.0 " —		
1913						
May 15	—	3	—	1.0 " —		

right hip for nine months. Status præsens: sluggish reaction of pupils to light, gait ataxic, Romberg marked, tactile hyperæsthesia over lower part of thorax, loss of sense of position in legs, both superficial and deep reflexes absent below waist, Charcot joint of right hip.

Treatment.—He had received practically no antisiphilitic treatment prior to November, 1910. In the first year of treatment he received sixteen intramuscular injections of bichloride of mercury, nine intravenous injections of salvarsan, a total of 2 gm., and six months mixed treatment by mouth. The result of this was to reduce the pleocytosis from 125 to 50 per c.mm. The effect on the Wassermann reaction in the cerebro-spinal fluid was not determined, because an insufficient quantity of fluid was employed. After the institution of intraspinal treatment with his own serum, the fluid became normal in six weeks. During this period a total of 1.4 gm. salvarsan was given in six intravenous injections

* Where both intravenous and intraspinal treatment were used, the date of the intravenous injection is indicated. The intraspinal injection was given on the following day.

and five intraspinal injections, a total of 60 c.c. of undiluted serum.* For over a year the fluid has remained normal and the patient has been practically free from pain. The Charcot joint has prohibited his return to his usual work, although he goes about very well. There has been no advance in his tabes.

TABLE II.

No. 213. O. W. A., Age 29. *Tabes Dorsalis*, 2½ Years; *Syphilis*, 9 Years.
Result—Improved.

Date.	Blood.		Cerebro-spinal Fluid.				Treatment.	
	Wassermann Reaction.	Cells per c.mm.	Noguchi Globulin.	Wassermann Reaction.			Intravenous.	Intraspinal Serum.
				Liver Antigen.	Cholesterol Heart Antigen.			
1911								
June 23 to Aug. 2	++						Salvarsan 5x0.2 gm.	
Aug. 5 to Aug. 10	++	110	—	0.2 c.c. ±				
Oct. 20 to Oct. 27	+	75	—	0.2 " ++			4x0.2 "	
Nov. 3 to Nov. 21	++			0.2 " ++			0.2 "	25 c.c. of 40%
Dec. 12 to Dec. 19	++	20	±	0.2 " ++			0.2 "	20 " of 50 "
	++	19	±	0.4 " ++			0.2 "	30 " of 70 "
							0.2 "	25 " of 40 "
1912								
Jan. 5 to Jan. 12	+	22	—	0.2 " ++			0.3 "	30 " of 50 "
Jan. 12 to Jan. 19	+	8	—	0.6 " ++			0.3 "	30 " of 50 "
Jan. 19 to Jan. 25	+	10	—	0.6 " ++			0.3 "	30 " of 50 "
Jan. 25 to Feb. 1	±	8	—	0.4 " ++			0.3 "	30 " of 50 "
Feb. 1 to Feb. 8	++	5	—	0.4 " ++			0.3 "	25 " of 40 "
Feb. 8 to Apr. 11	+	7	—	0.6 " ++			0.3 "	30 " of 50 "
Apr. 11 to Apr. 18	±	2	—	0.6 " ++			0.3 "	30 " of 40 "
Apr. 18 to May 2	++	10	—	0.6 " ++			0.3 "	30 " of 40 "
May 2 to May 16	++	8	—	0.6 " ++			0.3 "	30 " of 40 "
May 16 to May 19	+	10	—	0.6 " ++			0.3 "	30 " of 40 "
1913								
Feb. 22 to Mar. 7	++	1	—	0.8 " ++	0.6 c.c. ++	Neosal.	0.75 "	30 " of 40 "
Mar. 7 to May 3						"	6x0.75 "	
May 3 to June 20	++	3	—	1.0 " +	1.0 " ++	Salvarsan	0.5 "	

Case 213.—O. W. A. Had a chancre nine years previously; no secondaries. Treatment then consisted of pills, which were taken for two and a half years. For the past two and a half years he has had difficulty in voiding, increasing

* In the tables the amount of diluted serum which was injected at each treatment is shown, while in abstracts in the text the total amount of undiluted serum is given.

ataxia in walking for two years, marked pain and hyperæsthesia in lower back for a like period, occasional lightning pains in legs; disease rapidly advancing for past six months. Status præsens: pupils unequal, gait very ataxic, Romberg marked, band of hyperæsthesia around chest, marked hyperæsthesia in lumbar region, radial periosteal, patellar and tendo Achilles reflexes absent.

Treatment.—In the first four months of treatment, a total of 1.8 gm. of salvarsan was given in nine intravenous injections. This produced a drop in cells from 125 to 75, but had no effect on the Wassermann reaction in the cerebro-spinal fluid. Upon the institution of intraspinous injection of patient's own serum, there was an immediate drop in the number of cells. In the next seven months he received 4.3 gm. salvarsan in sixteen intravenous injections and fourteen intraspinous injections, a total of 184 c.c. of undiluted serum. Under this treatment the cells were reduced to practically normal in number, and the Wassermann reaction in the cerebrospinal fluid was much reduced in strength. Because the patient felt so much better he neglected to report for nine months, during which time the fluid remained practically the same. Then intravenous treatment was resumed and in four months' time 5.25 gm. neosalvarsan were given in seven injections. This was followed by a continued decrease in the strength of the Wassermann reaction in the cerebro-spinal fluid. The pains and lumbar hyperæsthesia have disappeared, but there is little change in the ataxia.

A marked beneficial effect was noted in both these patients when the intraspinous treatment was instituted, but the objection might be advanced that the improvement was due to the continuation of the salvarsan intravenously. To determine the effect of the intraspinous treatment, several patients were treated simply with intraspinous injections of serum from other patients who had received intravenous injections of salvarsan.

Case 557.—C. H. L. Thirty years before admission had a chancre followed ten months later by mucous patches in mouth, twenty-two years ago frontal neuralgia and gumma (?) of nose, ten years ago numbness of left arm, occasional shooting pains in legs and burning sensation in epigastrium; for three years occasional epigastric distress, nausea and vomiting, occipital headache for eighteen months, for six months daily vomiting and occasional abdominal girdle sensation; has lost much weight. Status præsens: much emaciated, pupils unequal, Argyll-Robertson irregular in outline, beginning optic atrophy, slight deafness, slight inco-ordination in hands, slight Romberg; anæsthesia over ulnar surface of arms, hypoæsthesia over left frontal region and chest, general hypoalgesia; conjunctival, pharyngeal and plantar reflexes absent, all deep reflexes except right tendo Achilles absent.

Treatment.—In three and a half months he received eleven intraspinous injections, a total of 130 c.c. undiluted serum, which was obtained from other patients. This treatment resulted in the reduction of all the abnormal constituents in the fluid nearly to normal. Then 1.5 gm. of salvarsan in five intravenous injections

TABLE III.

No. 557. C. H. L., Age 52. *Tabes Dorsalis*, 10 Years; *Syphilis*, 30 Years.
Result—Improved.

Date.	Blood.	Cerebro-spinal Fluid.			Treatment.	
	Wassermann Reaction.	Cells per c.mm.	Noguchi Globulin.	Wassermann Reaction.	Intravenous.	Intraspinal. Other Patients' Serum.
1911						
Oct. 25	—	42	++	0.2 c.c. ++		
30		41	++	0.2 " ++		25 c.c. of 40%
Nov. 6		40	++	0.2 " ++		20 " of 40 "
18		50	++	0.4 " ++		22 " of 40 "
Dec. 3	—	18	++	0.4 " ++		26 " of 50 "
12		17	++	0.2 " ++		30 " of 50 "
28		15	++	0.8 " ++		25 " of 40 "
1912						
Jan. 6		15	+	0.2 " ++		25 " of 40 "
12		10	+-	0.4 " ++		30 " of 50 "
23		14	++	1.0 " ±?		26 " of 50 "
31		16	+	1.0 " ±		30 " of 50 "
Feb. 7		17	+	1.0 " ±±		30 " of 50 "
Mar. 4	—	15	+	1.0 " ±±		
Apr. 10		10	±	1.0 " ±		
Apr. 16 to	—				Salvarsan 5x0.3 gm.	
May 14	—					
14		19	+	1.0 " ±		
Sept. 24	—	8	±	1.0 " —		
1913						
Mar. 28	—	5	—	1.0 " —		
June 21		2	—	1.0 " —		

was given with practically no effect on the fluid. The patient's condition was so much improved that he returned to work and received no further treatment. At the time of the last examination of the fluid, over a year after treatment was discontinued, the fluid was entirely normal. The patient has had no gastric disturbance for over a year and has gained 8 kilos in weight. The tabetic process has shown practically no advance since the treatment was started.

Case 675.—H. G. G. Denied syphilis; no antisyphilitic treatment previous to admission. Complained of shooting pain in legs for two and a half years, difficulty in walking for two years; compelled to give up his business because of pain and ataxia. Status præsens: unequal Argyll-Robertson pupils; gait,—combination of spastic and ataxic, marked Romberg; inco-ordination of arms and legs, cutaneous hypæsthesia and hypoaesthesia over left side of abdomen and outer side of left leg, delayed pain sensation below waist, loss of sense of position of legs; all superficial reflexes except left cremasteric absent, all deep reflexes absent.

Treatment.—In five and a half months he was given ten intraspinal injections, a total of 120 c.c. of undiluted serum. After eight treatments the cerebro-spinal fluid was normal. The symptomatic improvement, as well as the return to normal of the cerebro-spinal fluid was most striking. About three months

after his last treatment he died of lobar pneumonia. The cerebro-spinal fluid taken immediately after death was normal. Autopsy revealed changes in the cord typical of tabes dorsalis.

TABLE IV.

No. 675. H. G. G., Age 42. *Tabes Dorsalis*, 2½ Years; *Syphilis Denied*.
Result—Improved.

Date.	Blood.	Cerebro-spinal Fluid.			Treatment.
	Wassermann Reaction.	Cells per c.mm.	Noguchi Globulin.	Wassermann Reaction.	Intraspinal. Other Patient's Serum.
1912					
Mar. 18	±	35	±	0.6 c.c. ++	.
May 2	—				
5		41	+	0.6 " ++	30 c.c. of 40%
14		24	±	0.8 " ++	30 " of 40 "
21		17	±	0.8 " ++	30 " of 40 "
June 10	—				
20		14	±	1.0 " ++	30 " of 40 "
27		33	±	1.0 " ++	30 " of 40 "
July 9		12	±	1.0 " ++	30 " of 40 "
21		35	+	1.0 " ++	30 " of 40 "
30	—				
Sept. 19		14	—	1.0 " ±	30 " of 40 "
Oct. 1		8	—	1.0 " —	30 " of 40 "
19		7	—	1.0 " —	30 " of 40 "
1913					
Jan. 5	—	5	—	*1.0 " —	

Jan. 5. Died of lobar pneumonia. Autopsy showed typical tabes.

Case 565.—V. H. Had a "chancroid" without secondaries nine years before admission. Seven years ago dizzy attacks for six months; for the past three years has had shooting pains in the legs, occasional dizziness, difficulty in voiding

TABLE V.

No. 565. V. H., Age 42. *Tertiary Syphilitic Meningitis (Radiculitis)*; *Syphilis*, 9 Years.

Date.	Blood.	Cerebro-spinal Fluid.			Treatment.
	Wassermann Reaction.	Cells per c.mm.	Noguchi Globulin.	Wassermann Reaction.	Intraspinal. Other Patient's Serum.
1912					
Apr. 5	+ ±	124	++	0.2 c.c. ++	
10		64	++	0.2 " ±	30 c.c. of 40%
19		17	+	0.4 " ++	30 " of 40 "
28		30	+	0.4 " ++	30 " of 40 "
May 7		23	+	0.4 " ++	30 " of 40 "
Sept. 12†		4	—	Negative	

* Heart Cholesterin Antigen.

† Report furnished by Neurological Institute.

and loss of sexual power. Status præsens: pupils irregular, react slowly to light; areas of delayed pain sensation over legs, reflexes normal.

Treatment.—One year before admission he had received two intravenous injections of salvarsan, followed by a course of mercury inunctions and considerable potassium iodide by mouth. Before this treatment was started, he had 94 cells per c.mm. in the cerebrospinal fluid. Our first examination showed 124 cells, a heavy globulin and a strongly positive Wassermann reaction. He then had 48 c.c. of serum in four intraspinal injections. Four months later he was examined in another hospital and his spinal fluid reported as normal. Unfortunately, the patient would not continue under observation. The symptomatic improvement was less marked than the improvement in the pathological cerebrospinal fluid.

TABLE VI.

No. 1304. C. G., Age 54. Cerebro-Spinal Syphilis, 4 Years; Syphilis, 25 Years. Result—Improved.

Date.	Blood.	Cerebro-spinal Fluid.				Treatment.	
	Wassermann Reaction.	Cells per c.mm.	Noguchi Globulin.	Wassermann Reaction.		Serum from Other Patients.	
				Liner Antigen.	Cholesterol Heart Antigen.		
1913							
Jan. 10	—	86	++	0.8 c.c. ++	0.2 c.c. ++	30 c.c. of 40% after Neo.	0.9 gm.
23		21	++	1.0 " ++	0.4 " ++	30 " of 40 " "	0.9 "
31		12	++	1.0 " ++	0.4 " ++	30 " of 40 " "	0.9 "
Feb. 7		27	+	1.0 " +	0.4 " ++	30 " of 40 " "	0.9 "
16	±	24	+	1.0 " —	0.2 " ++	30 " of 40 " "	0.9 "
27		42	++	1.0 " —	0.4 " ++		
Mar. 7		20	++	1.0 " ±	0.2 " ++	30 " of 40 " after Sal.	0.5 "
21		26	++	1.0 " —	0.4 " ++	30 " of 40 " "	0.5 "
Apr. 4		26	++	1.0 " —	0.4 " ++	30 " of 40 " "	0.5 "
18		19	+	1.0 " —	0.4 " ++	30 " of 40 " "	0.6 "
May 2		20	+	1.0 " —	0.8 " ++	30 " of 40 " "	0.5 "
16		21	+	1.0 " —	0.8 " ++	30 " of 60 " "	0.5 "
30	—	14	+	1.0 " —	0.6 " ++	30 " of 50 " "	0.5 "

Case 1304.—C. G. Twenty-five years ago had a chancre, followed by secondaries. He took medicine by mouth for eight months. Four years ago pain and stiffness in left knee and pain in back, loss of sexual power for three years, for nine months increasing weakness of legs with difficulty in walking, and incontinence of urine and feces. Status præsens: pupils unequal, left reacts only slightly to light, distinct deafness, coarse tremor in hands, marked Romberg, static ataxia in legs, tendon reflexes much exaggerated, atypical Babinski, bladder distended so that catheterization was necessary.

Treatment.—In four and a half months patient received twelve intraspinal injections, a total of 153 c.c. of serum obtained from other patients. Five of the sera were obtained following neosalvarsan and seven following salvarsan treatment. At first there was a marked drop in cells, but after the third injection

tion the decrease in the degree of pleocytosis was only slight. There was, however, a steady influence on the Wassermann reaction and excess of globulin. At the same time there was a distinct improvement in the patient's general condition and in the control of the bladder, so that during the last two months of treatment he was able to go several days without catheterization or incontinence of urine.

These four cases illustrate very well that a definite and marked improvement in the condition of the cerebro-spinal fluid can be brought about by the intraspinal injection of salvarsanized serum alone, since none of the four patients had any other form of treatment during the course of intraspinal injections. The question naturally arose whether the intraspinal injection of normal serum alone might not have the same effect, and was investigated with patient R. d'H.

TABLE VII.

No. 674. R. d'H., Age 38. *Tabes Dorsalis*, 3 Years; *Syphilis*?

Date	Blood.	Cerebro-spinal Fluid.			Treatment.
	Wassermann Reaction.	Cells per c.mm.	Noguchi Globulin.	Wassermann Reaction.	Intraspinal.
1912					
Jan. 26	++	55	+	0.4 c.c. ++	Normal Serum. 26 c.c. of 50% 30 " of 50 " 30 " of 50 " 30 " of 40 " 30 " of 40 " 30 " of 40 " 30 " of 40 " 30 " of 40 " 30 " of 40 "
30		50	±	0.2 " ++	
Feb. 8		24	±	0.2 " ++	
19		25	±	0.4 " ++	
27		21	±	0.4 " ++	
Mar. 5		15	+	0.4 " ++	
12		23	+	0.4 " ++	
19		24	+	0.4 " ++	
26		50	+	0.2 " ++	
					Serum from other salvarsan-treated patients.
Apr. 12	++	46	+	0.4 " ++	30 c.c. of 40%
May 9		9	±	0.4 " ++	25 " of 40 "
June 22		34	±	0.6 " ++	30 " of 40 "
July 5		15	±	0.6 " ++	30 " of 40 "
16	±	21	±	0.6 " ++	

Case 674.—R. d'H. Gave no definite history of syphilitic infection, although he had chancroids on three different occasions. Three years ago he had difficulty in going down-stairs and shooting-pain in the legs, for two years difficulty in voiding and occasional incontinence of urine, loss of sexual power for one year. Eighteen months ago he had fifteen intramuscular injections of mercury. Status præsens: pupils unequal, react slowly to light, beginning optic atrophy,

slight ataxic gait, well marked Romberg, delayed pain sensation over trunk and legs, biceps and radial periosteal reflexes exaggerated, other deep reflexes absent.

Treatment.—In two months' time patient received eight intraspinal injections, a total of 93 c.c. of normal human serum. This was followed at first by a distinct reduction in the degree of pleocytosis, but at the end of the course of normal serum the cell count was as high as at the beginning. The globulin was persistently stronger than at the beginning and the Wassermann reaction in the fluid was but little influenced. Clinically the patient seemed somewhat improved. He was then given four intraspinal injections of salvarsanized serum, a total of 48 c.c. After the first injection there was a marked drop in the number of cells, but the cell count later rose somewhat. The globulin was persistently weaker. The Wassermann reaction in the fluid was also slightly weaker than it was at the end of the course of normal serum. More interesting was the diminution in the strength of the Wassermann reaction in the blood. This was confirmed on subsequent examination. Unfortunately the patient has refused to return for examination, although he has reported that he is working steadily at his usual occupation. In this patient there seemed to be some beneficial effect from the intraspinal injection of normal serum, which was less marked, however, than the injection of salvarsanized serum in the four patients previously cited, and also less marked in this patient than the injection of salvarsanized serum. When we consider that the salvarsanized serum has a definite antispirechætal action, it is reasonable to expect that its use intraspinal would be more effective than the injection of normal serum. The improvement in both the cerebro-spinal fluid and in the clinical condition of this patient indicates that the influence exerted by the intraspinal injection of human serum is beneficial rather than harmful. As there is a definite effect from intravenous injection of salvarsan, it would seem that the combination of intravenous and intraspinal treatment would act more rapidly than either alone. The following cases appear to illustrate this point.

Case 893.—F. B. Thirteen years before admission had three chancroids; no secondaries; took pills for one month. For past two years he has had shooting pains in the legs and stiffness of the knees, for six months difficulty in walking in the dark. Status præsens: pupils normal, gait slightly ataxic, moderate Romberg, area of cutaneous hypoæsthesia over perineum, deep reflexes in arms diminished, in legs absent.

Treatment.—In four months he received intravenously 8.2 gm. neosalvarsan and four intraspinal injections, a total of 48 c.c. of his own serum. The increase in the intensity of the Wassermann reaction after the treatment was started is worthy of note. The sudden drop in cells and the disappearance of the Wassermann reaction occurred after the first intraspinal injection. After the fluid was reduced to normal except for a slight increase in globulin, the treatment was continued intravenously because of the persistence of the Wassermann reaction in the blood serum. This case illustrates the point which we have frequently noticed, that if before treatment was started, large quantities of the fluid are required to obtain a positive Wassermann reaction, the reaction disappears fairly rapidly under treatment.

TABLE VIII.

No. 893. F. B., Age 34. *Tabes Dorsalis*, 2 Years; *Syphilis*, 13 Years.
Result—Improved.

Date.	Blood.	Cerebro-spinal Fluid.			Treatment.	
	Wassermann Reaction.	Cells per c.mm.	Noguchi Globulin.	Wassermann Reaction.	Intravenous.	Intraspinal Serum.
1912						
Oct. 18	+	40	±	1.0 c.c. +	Neosal. 0.75 gm.	
28	++				" 0.45 "	
Nov. 2		57	+	1.0 " ±±		
4	++	47	+	1.0 " ++	" 0.9 "	30 c.c. of 40%
12		13	+	1.0 " -	" 0.9 "	30 " of 40 "
18					" 0.9 "	
25	++				" 0.9 "	
Dec. 2	++	10	+	1.0 " -	" 0.9 "	30 " of 40 "
16					" 0.9 "	
17		2	±	1.0 " -		
	<i>Cholesterin Heart Antigen.</i>			<i>Cholesterin Heart Antigen.</i>		
1913						
Jan. 25		7	±	1.0 c.c. -		
Feb. 8	++	5	±	1.0 " -	" 0.9 "	30 " of 40 "
Mar. 14	++				" 0.75 "	
Mar. 28	++					
to					Salvarsan	
Apr. 25	++				3x0.5 "	
25	++	3	±	1.0 " -		
May 12	++					
to						
June 6	++				" 3x0.5 "	
20	±±	5	±	1.0 " -	" 0.5 "	

Case 605.—F. K. Absolutely denied history of primary or secondary syphilis; no antisyphilitic treatment. Three months before admission had three short attacks of dizziness; six weeks ago noticed that left pupil was larger than right. Status præsens: physical examination entirely normal except for dilated pupil and paralysis of accommodation in left eye.

Treatment.—In one year he received twenty intravenous injections of salvarsan, a total of 6.0 gm., and 3.6 gm. of neosalvarsan and 144 c.c. of his own serum in twelve intraspinal injections. During the first five months, when both intravenous and intraspinal treatment were given, there was a steady improvement in the condition of the cerebro-spinal fluid. Then for three months only intravenous treatment was given, and the condition of the fluid was practically stationary. After the intraspinal injections were resumed, the fluid again improved, so that at the end of the year after the beginning of treatment it was normal. Intravenous treatments were continued because of the persistent positive Wassermann reaction in the blood. This is the type of case which Nonne¹⁰ and Erb have described as usually going on to tabes or paresis. The distinct changes which were first found in the cerebro-spinal fluid indicate that the

TABLE IX.

No. 605. F. K., Age 27. Cerebro-Spinal Syphilis. Duration? No Previous Treatment. Result—Improved.

Date.	Blood.	Cerebro-spinal Fluid.			Treatment.	
	Wassermann Reaction.	Cells per c.mm.	Noguchi Globulin.	Wassermann Reaction.	Intravenous.	Intraspinal Serum.
1912						
Apr. 25	++	59	+	0.4 c.c. ++	Salvarsan 0.3 gm.	30 c.c. of 40%
May 2	++			0.6 " ++	" 0.3 "	30 " of 40 "
9	+				" 0.3 "	
23	+	14	±	0.6 " ++	" 0.3 "	30 " of 40 "
June 13	+	5	±	0.6 " ++	" 0.3 "	30 " of 40 "
20	+	6	±	0.8 " ++	" 0.3 "	30 " of 40 "
July 18	±	2	±	1.0 " ++	" 0.3 "	30 " of 40 "
26					" 0.3 "	
Aug. 15	±	2	±	0.8 " ++	" 0.3 "	30 " of 40 "
Sept. 12	±	6	±	0.8 " ++	" 0.4 "	30 " of 40 "
Oct. 3	±				" 2x0.3 "	
17	—				" 0.6 "	
Nov. 2		7	—	1.0 " ++		
22	+				" 0.6 "	
Dec. 12	—	8	—	1.0 " ++	Neosal. 0.9 "	
	Cholesterin Heart Antigen.			Cholesterin Heart Antigen.		
1913						
Jan. 3	++	4		1.0 " ++	" 0.9 "	30 " of 40 "
31	++	3	—	1.0 " ++	" 0.9 "	30 " of 40 "
Feb. 28	±±	4	—	1.0 " +	" 0.9 "	30 " of 40 "
Apr. 4	±±				Salvarsan 0.6 "	
25	+	2	±?	1.0 " —	" 0.5 "	30 " of 40 "
May 12	+					
to						
June 9	+				" 5x0.5 "	
16	++	0	—	1.0 " —	" 0.5 "	

patient was suffering from an active syphilis of the central nervous system, although he presented as physical signs only one dilated pupil. It seems reasonable to suppose that the treatment with the resulting return to normal of the cerebro-spinal fluid has resulted in an arrest of the syphilitic process. Probably by careful clinical examination, as well as examination of the cerebro-spinal fluids, many cases of tabes could be detected in this early stage and arrested by applying intensive and prolonged treatment. It is worthy of note that many of the tabetics give a history of diplopia or other transient eye disturbances several years before the onset of any other symptoms. It is important to recognize the disease in this early stage in order that it may not be allowed to progress until there is marked degeneration of the nervous tissue.

Case 903.—A. N. P. Eighteen years before admission had a chancre on the hand, followed by mucous patches in the mouth; medication by mouth for three years. Nine years ago 'gastritis' and marked lumbar hyperæsthesia to cold; shooting pains for four years, difficulty in voiding and occasional incontinence

of urine for four years; difficulty in walking in the dark and weakness of legs for six months. Status præsens: pupils unequal, react normally, gait slightly ataxic, slight Romberg; muscular hypotonus in arms and legs, band of hypoesthesia over lower chest, deep reflexes absent.

TABLE X.

No. 903. A. N. P., Age 44. *Tabes Dorsalis*, 9 Years; *Syphilis*, 18 Years.
Result—Improved.

Date.	Blood.		Cerebro-spinal Fluid.				Treatment.	
	Wassermann Reaction.	Cells per c.mm.	No-guchi Globulin.	Wassermann Reaction.			Intravenous.	Intraspinal Serum.
				Liver Antigen.	Cholesterol Heart Antigen.			
1912								
Dec. 3	++	44	±	0.6 c.c. ++			Neosal. 0.6 gm.	
5	++						" 0.75 "	
12	++						" 0.75 "	
19	++	28	±	1.0 " ++				30 c.c. of 40%
1913								
Jan. 9	++	10	±	1.0 " ++	0.6 c.c. ++		" 0.75 "	30 " of 40 "
16	++						" 0.3 "	
22	++	17	±	1.0 " ±	0.8 " ++		" 0.75 "	30 " of 40 "
Feb. 5	++	13	±	1.0 " ±	0.6 " ++		" 0.75 "	30 " of 40 "
19	++	13	±	0.6 " ++	0.4 " ++		" 0.75 "	30 " of 40 "
Mar. 12	++		±	0.8 " ++	0.8 " ++		" 0.75 "	30 " of 40 "
27	++	7	±	1.0 " +	0.6 " ++		" 0.75 "	30 " of 40 "
Apr. 23	++	6	±	1.0 " +	0.8 " ++	Salvarsan	0.5 "	30 " of 40 "
May 7	++		±	1.0 " —	1.0 " +±		" 0.5 "	30 " of 40 "
21	++						" 0.5 "	
June 18	+	8	±	1.0 " —	1.0 " +±		" 0.5 "	30 " of 40 "

Treatment.—In seven months fourteen intravenous injections, a total of 6.9 gm. neosalvarsan and 2 gm. of salvarsan were given, and in addition 120 c.c. of serum in ten intraspinal injections. The improvement in the cerebro-spinal fluid in this case was not as rapid as in some others, but it has been fairly continuous. The clinical improvement has been most marked, especially the diminution in bladder symptoms. There has been a very noticeable improvement in the bladder symptoms in many of the patients who have been treated intraspinaly.

Case 152.—O. F. Illustrates both the results of intraspinal treatment and the necessity of continuing some form of treatment until the cerebro-spinal fluid is normal. He had a chancre ten years before admission; at that time he took pills for three months. Four years ago injured his back; six months later began to have shooting pains in legs; three years ago band-like sensation below knees and difficulty in walking. With Fraenkel re-education, there has been some improvement in walking. Status præsens: pupils unequal, irregular, poor reaction to light; gait, markedly ataxic, marked Romberg, loss of sense of position in legs; hypoesthesia below level of second intercostal space, superficial and deep reflexes all absent.

TABLE XI.

No. 152. O. F., Age 29. *Tabes Dorsalis*, 3½ Years; *Syphilis*, 10 Years. Result—Improvement, Relapse, Improvement.

Date.	Blood.		Cerebro-spinal Fluid.				Treatment.	
	Wassermann Reaction.	Cells per c.mm.	No-guchi Globulin.	Wassermann Reaction.			Intravenous.	Intraspinous Serum.
				Liver Antigen.	Cholesterolin Heart Antigen.			
1911								
Apr. 4	++	27	++	0.2 c.c. —			Salvarsan	
May 18	+						" 5x0.2 gm.	
19		39	++	0.2 " +				
Oct. 13	+						" 0.2 "	
20	±	9	+	0.2 " ±			" 0.2 "	17 c.c. of 40%
27								
to								
Nov. 17							" 4x0.2 "	
20		10	+					
1912								
Apr. 9	—	14	±	0.4 " ++			" 0.3 "	30 " of 40 "
15							" 0.3 "	
23	±	3	+	0.4 " ++			" 0.3 "	30 " of 40 "
30	±						" 0.3 "	
May 7	±	6	+	0.4 " ++			" 0.3 "	30 " of 40 "
Sept. 23	±	19	+	0.6 " ++			Neosal. 0.9 "	
Oct. 8	±						" 0.9 "	
	Cholesterolin Heart Antigen.							
1913								
Jan. 31	+±	70	+	0.4 " ++	0.2 c.c. ++		" 0.75 "	
Feb. 7	+±	57	±	0.4 " ++	0.4 " ++		" 0.9 "	30 " of 40 "
14	+±	28	±	0.6 " ++	0.4 " ++		" 0.9 "	30 " of 40 "
28	+±	17	±	0.6 " ++	0.4 " ++		" 0.9 "	30 " of 40 "
Mar. 14	+	14	—	0.4 " ++	0.4 " ++		" 0.75 "	30 " of 40 "
27	++	7	+	1.0 " ++	0.6 " ++		" 0.75 "	30 " of 40 "
Apr. 9	+±	7	+	1.0 " ±	0.6 " ++		" 0.9 "	30 " of 40 "
June 3	++	5	±	1.0 " +	0.8 " ++		Salvarsan	
							" 0.5 "	30 " of 40 "
12	++						Neosal. 0.9 "	
17	++	2	—	1.0 " ±	1.0 " ++		Salvarsan	
							" 0.5 "	30 " of 40 "

Treatment.—In the first year he received 22 gm. salvarsan in eleven intravenous injections, which resulted in slight reduction in the degree of pleocytosis, a moderate reduction in globulin, and, as far as could be determined, little if any effect on the Wassermann reaction in the cerebro-spinal fluid. Then in one month's time he had 1.5 gm. salvarsan in five intravenous injections plus three intraspinous injections, a total of 36 c.c. of his own serum. This resulted only in a reduction of the pleocytosis. For the next eight months he refused to have systematic treatment. In January, 1913, the pains became more severe, and about the end of the month he developed diplopia. Examination of the cerebro-spinal

fluid revealed a more marked pleocytosis than on any previous examination. In the next two months systematic treatment, consisting of seven intravenous injections, a total of 5.8 gm. neosalvarsan, and six intraspinal injections of his own serum, a total of 72 c.c., resulted in a reduction in cells to nearly normal, and a marked decrease in the intensity of the Wassermann reaction in the cerebro-spinal fluid. At the same time the pains and diplopia disappeared. During two months' rest from treatment, the fluid showed slight improvement, and with the resumption of treatment, the fluid is undergoing a striking change for the better. During the past four months the patient has been freer from pain than during any similar period in the past five years. The symptomatic relapse and the increase in the signs of the active pathological process, as shown by the cerebro-spinal fluid in this patient, are in striking contrast to the subsequent course of the disease in those patients whose cerebro-spinal fluid has been rendered entirely normal by treatment.

Case 113.—W. L. S. Had a chancre with secondary eruption twenty-three years previously. At that time he took pills for three years. Seven years ago began to have shooting pains in the legs; five years ago transitory diplopia; three years ago return of the diplopia which has persisted; gradually increasing difficulty in voiding for past five years. Status præsens: signs of aortitis, pupils react slowly to light, paralysis of external rectus muscle of left eye, beginning optic atrophy, slight Romberg, deep reflexes in arms diminished, in legs absent.

Treatment.—In the first ten months of treatment he had seven intravenous injections of salvarsan, a total of 1.8 gm., and six months' mixed treatment. This resulted in a reduction in cells from 72 to 36 per c.mm., and a moderate decrease in globulin. In the next three months he received 2.5 gm. salvarsan in nine intravenous injections and nine intraspinal injections of his own serum, a total of 109 c.c. At the end of this course of treatment, the cerebro-spinal fluid was normal except for a trace of globulin. Because of the persistence of a positive Wassermann reaction in the blood, intramuscular injections of mercury were given, but could not be tolerated long. Occasional lumbar puncture showed that the cells were increasing slightly in number, and systematic intravenous treatment with neosalvarsan was given. After five treatments, a total of 3.3 gm., it was found that the spinal fluid again showed a positive Wassermann reaction with 0.8 c.c. This returning reaction cannot be attributed to the increased sensitiveness of the cholesterin-heart antigen, because the reaction was negative in January with the same antigen. After two more injections of neosalvarsan and four of salvarsan, the Wassermann reaction was again negative in the cerebro-spinal fluid, but the pleocytosis had increased. This patient has shown less symptomatic improvement than any of our other patients. After a course of treatment there has been some decrease in pain with periods of a week or more without pain, but no permanent relief has been obtained. The return of the abnormal constituents in the cerebro-spinal fluid indicates that the syphilitic process is still active in some part of the cerebro-spinal axis. The disappearance of these evidences of inflammation under combined intravenous and intraspinal treatment, and their reappearance under intravenous treatment alone point to the advantage of the combined method in this patient at least.

TABLE XII.

No. 113. W. L. S., Age 52. *Tabes Dorsalis*, 7 Years; *Syphilis*, 23 Years.
Result—Improvement; Relapse.

Date.	Blood.	Cerebro-spinal Fluid.			Treatment.	
	Wassermann Reaction.	Cells per c.mm.	Noguchi Globulin.	Wassermann Reaction.	Intravenous.	Intraspinal Serum.
1911						
Mar. 7	++	72	++	0.2 c.c. —	Salvarsan 0.35 gm.	
11	++				" 0.5 "	
28	++	27	++	0.2 " —	Mixed treatment by mouth	
Apr. 4 to	+				Salvarsan	
May 22	++				5x0.2 "	
Oct. 5	++					
Oct. 7 to	±					
Nov. 4	±					
5		36	+	0.2 " —		
1912						
Jan. 5	+	24	++	0.8 " ++	" 0.3 "	30 c.c. of 40%
13	+	30	+	0.8 " ++	" 0.3 "	28 " of 40 "
21	++	10	++	0.8 " ++	" 0.3 "	26 " of 50 "
27	++	8	++	1.0 " ++	" 0.3 "	25 " of 40 "
Feb. 3	+	28	+	0.8 " ++	" 0.3 "	30 " of 50 "
10	++	5	++	0.8 " ++	" 0.3 "	30 " of 50 "
Mar. 16	+	9	+	1.0 " +	" 0.3 "	30 " of 40 "
23	+	3	+	0.8 " ++	" 0.2 "	30 " of 40 "
Apr. 6	±	3	±	1.0 " —	" 0.2 "	30 " of 40 "
13	+	3	±	1.0 " —	" 0.3 "	
May 27 to					6 injections Hg. salicylate intra-muscularly	
July 8					Neosal. 0.9 gm.	
Sept. 13	+	2	±	1.0 " —	" 0.9 "	
21	±					
Nov. 23		6	+	1.0 " —		
	<i>Cholesterin Heart Antigen.</i>			<i>Cholesterin Heart Antigen.</i>		
1913						
Jan. 25	++	9	±	1.0 c.c. —	" 0.6 "	
Feb. 8	++				" 0.6 "	
22	++				" 0.6 "	
Mar. 8	++				" 0.75 "	
22					" 0.75 "	
Apr. 5	++	7	+	0.8 " ±	" 0.6 "	
17	++				" 0.75 "	
May 3	++				Salvarsan 0.4 "	
17	++				" 0.3 "	
31	++				" 0.4 "	
June 14	++	12	+	1.0 " —	" 0.4 "	

In addition to the twelve cases above cited in detail, the results of the treatment of twenty more cases by the combined method are shown in Table XIII, where the findings before treatment, amount and nature of treatment, and the changes in the cerebro-spinal fluid

TABLE XIII.

Name.	Diagnosis.	Before Treatment.				Treatment.				After Treatment.																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																			
		Duration of Symptoms.	Duration of Syphilis.	Cerebro-spinal Fluid.			Blood Wassermann Reaction.	Cells per c.mm.	Globulin.	Wassermann Reaction.	Salvarsan.	Neosalvarsan.		Serum Intra-spinously.	Blood Wassermann Reaction.	Cerebro-spinal Fluid.																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
				Cells per c.mm.	Globulin.	Wassermann Reaction.						Total Amount.	No. of Injections.			Total Amount.	No. of Injections.	Total Amount.	No. of Injections.	Cells per c.mm.	Globulin.	Wassermann Reaction.																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																							
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resulting from this treatment are given. These patients have, for the most part, been treated intravenously every two weeks with full doses of salvarsan or neosalvarsan, followed the next day by intraspinous injections of 30 c.c. of 40 per cent. serum. It is evident that most of the cases under this form of treatment show rapid alterations in the pathological picture in the cerebro-spinal fluid. Not only is there a rapid diminution in the pleocytosis, but the Wassermann reaction in the fluid usually diminishes steadily in intensity under the influence of the combined treatment. The globulin excess usually disappears more slowly than do the other two abnormal constituents, although this is not always the case.

TABLE XIV.

Change in Strength of Wassermann Reaction in the Cerebro-Spinal Fluid Resulting from Treatment.

Total Cases.	Strength of Wassermann Reaction before Treatment.	Strength of Wassermann Reaction after Treatment.								Duration of Treatment before Reaction Became Negative.	
		0.1 c.c.	0.2 c.c.	0.4 c.c.	0.6 c.c.	0.8 c.c.	1.0 c.c.	Negative.	Negative %.	Combined.	Intraspinous Alone.
3	0.1 c.c.	1	1	1					0		
10	0.2 "		1		3	1	3	2	20%		{ 1 mo. 4½ mos.
6	0.4 "			1			3	2	33 "	{ 1½ mos. 1 yr.	
5	0.6 "						3	2	40 "	{ 4 mos. 1½ "	4 "
3	0.8 "						1	2	66 "	{ 1 yr. 1 mo.	
5	1.0 "							5	100 "	{ 1 " 8 mos. 1½ " 1 yr.	
Totals, 32		1	2	2	3	1	10	13	41%		

A summary of the change in the intensity of the Wassermann reaction in the cerebro-spinal fluid of the thirty-two cases presented in this communication is given in Table XIV. The table is arranged so that in reading from above downward, the strength of the reaction before treatment can be determined, while in reading from left to right, the strength of reaction after treatment is indicated. For example, among ten cases which gave a completely positive

reaction at 0.2 c.c., one remains at that point, three have changed to 0.6 c.c., one to 0.8 c.c., three to 1.0 c.c. and two have become entirely negative. Only three out of the thirty-two have failed to decrease in intensity as a result of the treatment, and two of these have been under treatment only two months; the other is an early general paresis. In thirteen of the cases (41 per cent.) the Wassermann reaction has become completely negative, and in ten others (31 per cent.) the reaction has become so much weaker that 1.0 c.c. of cerebro-spinal fluid is required to give fixation. We have frequently noticed that when reactions have moved to 1.0 c.c., only a comparatively small amount of treatment is required to render them entirely negative, so that probably with two months' more treatment, between 60 and 70 per cent. of the cases will show a negative reaction in the cerebro-spinal fluid. The time required to render the reaction negative is given in a special column. In three of the cases the reactions became negative under intraspinal injection alone; the others had combined intravenous and intraspinal treatment. In those cases which required a year to show negative reactions, the treatment was less intensive than is now applied. Another point brought out by the table is that the stronger the initial strength of the reaction in the cerebro-spinal fluid, the more difficult is it to reduce the fluid to normal, so that by determining the strength of the reaction before treatment, we have a rough prognostic index; that is, a patient with an initial reaction at 0.1 c.c. or 0.2 c.c. will probably require more treatment than one with a weaker reaction.

Our patients with clinical pictures which suggest a possibility of paresis and very strongly positive Wassermann reactions have shown a rapid decrease in pleocytosis, but a slower response in their Wassermann reactions. When one considers the anatomical difficulties in reaching the myriads of spirochætæ that are present in the brain in this condition, he is impressed with the necessity of prolonged and vigorous treatment.

Still another point brought out by the work here presented is that the treatment of the chronic syphilitic diseases of the central nervous system is not the administration of any definite amount of salvarsan, mercury or iodides. Each case presents a slightly different problem and must be considered individually. Some respond much

more rapidly than others, but the object in all should be to obtain a persistently normal cerebro-spinal fluid. Of course, we cannot at present state with certainty that a normal cerebro-spinal fluid assures us that there will be no further progress of the degenerative process. All we can say is that as long as the cerebro-spinal fluid gives evidence of a specific pathological process in the central nervous system, and we have specific therapeutic measures which will remove that evidence, these measures should be applied. Only when the fluid has become normal are we justified in discontinuing the treatment, and observations of the fluid should be continued, so that at the first evidence of relapse treatment may be resumed.

The method of intraspinal injection is not presented as a substitute for any of the accepted forms of treatment, but only as an aid in attacking these severe infections. We feel that there is definite evidence that this form of treatment has a curative action on the syphilitic process, and that therefore its combination with intensive intravenous treatment will be of benefit when specially intensive treatment is required, as in rapidly advancing tabes or paresis, or where the disease has resisted the usual forms of treatment.

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TREATMENT OF PNEUMONIA BY MEANS OF SPECIFIC SERUMS.*

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It is now over twenty years since the first attempts were made to treat patients suffering from acute lobar pneumonia by means of serum obtained from animals immunized to pneumococcus. In 1904 Anders¹ collected the reports of 535 cases treated in this way. He concluded that the practical results obtained by the use of anti-pneumococcus serum did not warrant its general use. Nevertheless, the theoretical study and practical application of antipneumococcus serum have not ceased. Although it has been impossible to demonstrate any striking lowering of mortality in a large series of cases, yet certain good clinical observers have felt that in certain individual instances the results were striking. The experimental evidence, moreover, has been sufficient to inspire hope in the minds of many. It has become evident, however, that the problem can be solved only by more refined and critical methods than those previously employed.

It has been known since 1891 that susceptible animals may be rendered resistant to the action of pneumococci by the injection of increasing and properly spaced doses of pneumococci, beginning with the dead organisms. Moreover, it was early shown that if a very small amount of the serum of the immunized animals is injected into a second animal, this animal for a short time is also immune. This experiment is so striking and fundamental that it is

* Read in the symposium on Serums and Vaccines at the joint meeting of the Sections on Pharmacology and Practice of Medicine of the American Medical Association at the Sixty-Fourth Annual Session, held at Minneapolis, June, 1913.

1. Anders, J. M.: Serum Treatment of Pneumonia, THE JOURNAL A. M. A., Dec. 10, 1904, p. 1777.

little wonder that the efforts to find methods for using this serum therapeutically in man have been persisted in. The results of treatment in animals, however, as contrasted with prevention or protection, have not been so satisfactory. While a very small amount of serum will usually protect an animal from a large dose of bacteria given with the serum or a very short time afterward, even a large amount of serum will usually not cure the animal after infection is well advanced. Evidence is not lacking, however, that even in animals, such immune serums may have curative as well as protective action. Efforts in treatment have usually been attempted in rabbits and mice, which are extremely susceptible to pneumococcus infection and in which the infection runs a very rapid course.

When injections of pneumococci are made directly into the lungs of guinea-pigs, the infection runs a slower course, and Neufeld and Ungermann² have shown that in such cases, if injections of even small amounts of serum are made as late as three hours following the infection, recovery occurs in a large proportion of the animals. It is possible, therefore, that in man, in whom the pneumococcus infection runs a much more chronic course and tends to become localized, the serum might be efficacious in treatment, if a suitable method for its administration should be discovered. During the past few years, moreover, experimental studies have shown possible reasons why immune serums have not proved more efficacious in the past, and have indicated methods by which they may be made more efficacious in human infection.

In titrating immune serum against varying doses of pneumococci by injection into mice, Neufeld and his assistants have shown that a certain amount of serum, in relation to the body-weight, is required to protect. This amount protects against many times the lethal dose. On the other hand, a slightly smaller dose may not protect at all, even against only a very small multiple of the minimal lethal dose. In other words, such a serum does not obey the law of multiple proportions and to be efficacious, even against a very mild infection, it must be present in the body in a given concentration. This concentration they have called the *Schwellenwert*, or threshold concentration. Reckoning from their experiments on mice, they

2. Neufeld and Ungermann: Berl. klin. Wchnschr., 1911, xlviii, 717, 1010.

estimate that in man the curative dose of the variety of serum tested by them must be at least 75 c.c. Since curative injections in man, however, are ordinarily not made until many hours after the infection has started, it is probable that in most cases the doses must be much larger than this. It is evident, therefore, that one reason why antipneumococcus serum has not been more efficacious in the past is that it has not been administered in sufficiently large doses.

On the other hand, experiments performed by Dochez have shown that there is a maximum degree of infection against which no amount of serum, however large, is able to protect. It would therefore seem that one of the factors of the protective mechanism must be supplied by the body, and that, when the infection is very great, a sufficient number of immune bodies may be supplied by the administration of serum, but the body cannot react to a sufficient extent to adequately supply this second factor. This suggests that in order to obtain results from serum, it should be administered early, before the infection has reached too extreme a grade, beyond which no amount of serum can be effective, and also offers a possible explanation of why in certain cases, such as the one which I shall mention, the serum seems to have absolutely no effect.

Further studies indicate still another and more important reason why antipneumococcus serum has not proved of value in the past, and explain why even the administration of very large doses early in the disease is likely to prove of value in only a small proportion of cases. In the past, antipneumococcus serum has been administered indiscriminately in all cases of pneumonia, no effort being made to determine in the individual case the nature of the bacterium causing the infection. It has long been known that characteristic lobar pneumonia may be caused by a number of other organisms besides the pneumococcus, such as the streptococcus and influenza bacillus. It is well recognized that an antipneumococcus serum cannot be effective in case the disease is due to an organism other than the pneumococcus, since such serums are as rigidly specific in their immune reactions as is antidiphtheritic serum for diphtheria toxin. It must be granted, however, that a large majority of the cases of typical lobar pneumonia are due to pneumococcus, so that if such a serum were efficacious against all such cases, the results of its administration would be manifest.

Neufeld found an antipneumococcus serum prepared by him by the immunization of a horse with a given race of pneumococci effective against the race of pneumococci used for immunization and also against certain other races obtained from cases of pneumonia; but against still other races of typical pneumococci, he found that it had practically no effect. In order to overcome this difficulty, Neufeld had recourse to an expedient previously employed in immunization work, that is, the preparation of a serum by the inoculation of a number of different races of pneumococci, hoping thus to prepare a polyvalent serum efficacious against practically all the races likely to be encountered. This has seemed to me a crude method of dealing with the problem and one not likely to advance our knowledge of the exact conditions concerned.

Without going into the details of the methods which have been described elsewhere by Dochez, it may be stated that the pneumococci isolated from cases of pneumonia which I have studied may be divided into four groups. The organisms belonging to each of the first two groups are specific, so far as their immune reactions are concerned. An immune serum produced by the injection of a horse with a race belonging to Group 1 has a specific action against all the members of Group 1, but has no effect on the organisms of any of the other groups. In like manner, an immune serum produced by the injection of a horse with a pneumococcus belonging to Group 2 is protective against all the other members of this group, but has no effect against the members of any of the other groups. In Group 3 are included the organisms of the so-called *Pneumococcus mucosus* type. These organisms have very large capsules and produce a sticky exudate in animals. So far it has not been possible to produce an active immune serum against this variety, though an effective active immunity may be obtained. An immune serum produced by a member of any of the other groups has no protective power for any race of this group. In Group 4 are included all the races against which Serums 1 and 2 are not effective and which, from their other properties, do not belong in Group 3. Animals may readily be immunized to any member of this group, and the serum of the immunized animal is protective against the race used for immunization. In no instance, however, has this serum been

found effective against any other race of this group or against the organisms of the other groups. So far as cultural and morphologic characters are concerned, no constant group differences have been discovered between the members of Groups 1, 2 and 4. By means of the agglutination reaction of the various races, however, it has been found possible to group them in exactly the same manner as by protection experiments.

It has become evident, therefore, that while a large majority of cases of pneumonia are due to pneumococcus, so far as immune reactions are concerned the cases of pneumococcus pneumonia are caused by organisms of at least four different types, and, from the point of view of specific therapy, this is equivalent to saying that they are due to at least four organisms. In seventy-four cases of pneumonia studied the number of cases found to be due to organisms belonging to each of four groups is shown in Table 1.

TABLE 1.

Classification of Seventy-Four Cases of Pneumonia.

Type of Organism	Number of Cases	Percentage
1	35	47
2	13	18
3 (mucosus)	10	13
4 (heterogeneous)	16	22

It is now evident that in studying the effect of an immune serum on patients with pneumonia, but slight conclusions can be drawn from its indiscriminate employment in all cases. First, we must know the type of organism used for its production, and second, it must be employed only in cases due to organisms of this type. So far, it has been possible to produce a serum of high protective power against organisms of Type 1, and a second serum, only slightly less efficacious, against organisms of Type 2. As previously stated, it has been impossible to produce a serum active against organisms of Type 3. To produce a serum active against organisms of Type 4, it would be necessary to immunize an animal against every obtainable race of this group. This would not be practicable. So far as the treatment of pneumonia is concerned, however, this is of minor importance, since our observations indicate that pneumonia due to organisms of Type 4 is of relatively slight severity, and most

of the patients recover without any specific treatment. This is shown in Table 2.

TABLE 2.

Mortality.

Cases Due to	No. Patients	Died	Per Cent.
Type 1	34	8	24
Type 2	13	8	61
Type 3	10	6	60
Type 4	15	1	7
Total	72	23	32

At present, therefore, the problem has resolved itself into treating the cases of pneumonia due to organisms of Type 1 and Type 2 with Serum 1 and Serum 2. In order to treat the individual case, however, it is necessary to have a method of determining very promptly, after the patient comes under observation, the type of organism concerned. It has been found possible to do this by using the following method: When a patient with pneumonia is admitted to the hospital, a culture is immediately made from the blood and also one from a portion of sputum coughed up from the lung, or when this is not obtainable, a culture is made directly from the lung by the insertion of a needle. This procedure seems to be without danger. When there are large numbers of virulent organisms in the sputum, a culture may be most rapidly obtained by injecting the sputum into the abdominal cavity of a mouse. After four or five hours the peritoneal cavity may be washed out with salt solution and the cells thrown down in the centrifuge; a suspension of the organisms is thus obtained. However the culture is obtained, the agglutination test is at once applied. If the organism fails to agglutinate with either Serum 1 or Serum 2, it is, of course, useless to undertake serum treatment. If, however, one of the serums agglutinates the organism, treatment may be commenced at once with the appropriate serum. Later the organisms have been studied by testing the protective power of the serum, and in all cases the protection test has corresponded with the agglutination test.

We have now shown certain reasons why the use of immune serum in the past has not been efficacious, and methods have been described whereby, theoretically at least, such serum may be ren-

dered effective. It may be assumed that given serums of high protective power against organisms of Groups 1 and 2, the early determination of the nature of the infecting organism and the early administration of large doses of the appropriate serum should have a favorable influence on the course of the disease. So far it has been possible to test this assumption on only a comparatively small number of cases, since the work so far has been largely preliminary and done with a view to establishing the practical application of the serum on a sound experimental basis.

Serum has now been administered in twenty cases of pneumonia. In one of the first cases a further study of the organism showed it to be of Type 4, so the use of serum was discontinued. In two cases the patients were admitted in the last stages of the disease and the serum was administered only a few hours before the patients died. Seventeen cases have therefore been treated with the serum. Of these, fourteen were due to organisms of Type 1 and three to organisms of Type 2.

The method of administration of the serum was as follows: On admission, 2 c.c. of serum were injected subcutaneously to discover if the patient was hypersensitive. As soon as the type of organism was determined, from 50 to 100 c.c. of the serum, diluted one-half with salt solution, were injected intravenously. The condition of the patient served as a guide in the later treatment. Usually the serum was not administered oftener than every twelve hours. The patients treated received totals of from 190 to 460 c.c. of serum, except one, who received a total of 700 c.c. of serum. Treatment was commenced on the third day in six cases, the fourth day in four cases, the fifth day in three cases and the sixth day in four cases.

The patients treated were all seriously ill, the most severe cases we have had during the winter. In the fourteen cases due to *Pneumococcus* 1, all of the patients recovered but one, and in the three cases due to Type 2, one patient died. This patient, however, objected to the treatment and would not allow its continuation, so it was not thoroughly carried out. When we consider that the mortality among the cases due to Types 1 and 2 is very high, the result is certainly not discouraging. The number of cases is much too small,

however, for any conclusions to be drawn from the results as regards mortality.

At present conclusions as to the efficacy of the serum must be based on other criteria. Let us first consider the effect on the clinical course of the disease. Temperature curves are not here presented, since erroneous conclusions may be drawn unless all the curves are presented, and in the present instance this is impossible. Following practically all the injections, a reaction has occurred. The temperature usually rises, and then falls, but does not necessarily remain low. In two instances the rise of temperature has been marked. In other cases the rise of temperature following the injection was only a degree or so. In all the cases except the fatal ones, the serum has apparently had an ultimate favorable effect in lowering the temperature and shortening the course of the disease, though, of course, this is a very difficult matter of which to be absolutely sure. In no case was one injection of the serum sufficient to bring on a crisis. All the patients seemed to feel better following the injection of the serum, and in a number of cases the apparent lessening in the degree of intoxication was very manifest.

When the treatment was commenced early, no extension of the involvement of the lung occurred. On the other hand, however, there was no special tendency in the treated cases for the lung lesion to resolve rapidly. If anything, there seemed to be a tendency for resolution to be delayed in these cases. This has been noted by others in certain cases treated by serum. One of the patients developed a pneumothorax with empyema. Operation was performed and recovery ensued. Fortunately, this was not one of the patients in whom the lung was punctured for culture. Three patients later developed signs of fluid, and aspiration of small amounts of fluid was performed. The fluid in these cases was sterile and operation was not necessary.

More important than the foregoing criteria, however, in indicating an effect of the serum, are the following observations, since they have depended solely on objective procedures: First to be mentioned is the effect of the serum on the organisms in the blood. In eight cases pneumococci were isolated from the blood before the treatment was commenced. In all cases blood-cultures were made before each

treatment, and in all of these cases after one treatment and before the second (or within from eight to twelve hours) the blood had become sterile. The conclusion seems justified, therefore, that one large dose of active serum given intravenously is sufficient to sterilize the blood. It also seems certain that if the organisms are not present in the blood, the administration of the serum will prevent their invasion.

Second, in a previous study of the protective substances in the blood-serum of patients with pneumonia,³ it has been shown that, as a rule, the appearance of protective substances in the blood, when demonstrable, coincides rather sharply with the period of critical fall in temperature and the disappearance of symptoms. Before the crisis they are not present in the blood in any measurable degree.

A similar study has been made by Dochez of the protective substances in the serum in a number of the cases of pneumonia treated with serum. In all the cases studied, it has been possible to demonstrate the appearance of such substances in considerable amounts in the serum very shortly following the administration of one dose of the immune serum, even when this serum has been administered early in the disease, at a period when such protective substances are otherwise never present. These substances persist, and in case they play a part in the mechanism of recovery, as was concluded from the previous study, it is evident that their appearance indicates a favorable action of the immune serum.

The results obtained, therefore, from the clinical and laboratory study of this series of cases of pneumonia treated by the injection of large amounts of appropriate serum, seem to indicate that a method has been devised for the successful specific treatment of at least a portion of the cases of acute lobar pneumonia.

The mode of action of immune serum in these cases is still obscure. We have found no evidence of a bactericidal action of this serum, either *in vitro* or *in vivo*. The evidence that the effect is due, to any considerable degree, to the presence of bacteriotropic or opsonic substances is still inconclusive. The effect on the patients seems to in-

3. Dochez, A. R.: The Presence of Protective Substances in Human Serum during Lobar Pneumonia, Jour. Exper. Med., 1912, xvi, 665, abstr., THE JOURNAL A. M. A., Dec. 7, 1912, p. 2094.

dicating that the effect to some extent, at least, may be due to the presence of antitoxic substances. Until the toxin responsible for the symptoms in pneumonia is isolated, however, the final proof of antitoxic action cannot be demonstrated. It has been shown,⁴ however, that when living pneumococci are dissolved in bile, the resulting solution, when injected into rabbits and guinea-pigs, is very toxic. By means of experiments on animals, which will be reported elsewhere, it has been possible to demonstrate that the toxic action of such an extract may be neutralized by the addition of proper amounts of the corresponding immune serum. Such extracts have also been found to be lytic for red blood-corpuscles, and it has been possible to inhibit this lytic effect by means of the corresponding immune serum. It is therefore evident that in case this toxin plays a rôle in the symptoms of this disease, the action of the serum in man may be due, in part at least, to its antitoxic properties.

SUMMARY.

Antipneumococcus serum, in order to be effective, must be given early, in large amounts. Since pneumococci, so far as their immunologic properties are concerned, are divided into distinct groups, it is necessary in any case to administer the serum active against the group to which the organism infecting this case belongs. Highly active serums against two groups of pneumococci have been obtained and treatment has been carried out in a small number of cases. The effect in these cases has been favorable and the mortality has been very low. In judging of the value of the serums, however, other facts are considered of more importance; namely, first, that bacteria in the blood are destroyed, and second, that immune substances, which ordinarily appear in the blood about the time of crisis, appear in the treated cases very soon after the administration of the serum. Lastly, there is some experimental evidence that part of the action of the immune serum may be antitoxic.

4. Cole, Rufus: Toxic Substances Produced by Pneumococcus, *Jour. Exper. Med.*, 1912, xvi, 644, abstr., *THE JOURNAL A. M. A.*, Dec. 7, 1912, p. 2094.

A STUDY OF THE CEREBROSPINAL FLUID IN ACUTE POLIOMYELITIS.*

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York.)

In a paper published last year Draper and Peabody¹ reported on the examination of the cerebrospinal fluid in sixty-nine cases of acute epidemic poliomyelitis in the Hospital of The Rockefeller Institute for Medical Research. The present study deals with their findings and, in addition, with the results of the examination of fifty-seven more cases that were admitted to the hospital during the summer of 1912. The purpose of this study was to ascertain whether the examination of the spinal fluid may be of aid in early diagnosis and whether the abnormalities present have any relation to the severity of the disease, the localization of paralysis, and the prognosis.

In all, 362 examinations have been made in 126 cases. The points to which attention was paid were the macroscopic appearance of the fluid, the number and type of cells present, the presence of globulin, and the power of reducing Fehling's solution. In all cases lumbar puncture was performed as soon after admission as possible, and at varying intervals afterwards, in some cases on successive days. The period covered extends from the preparalytic stage to the twelfth week after onset. The amount of fluid withdrawn varied from a few cubic centimeters to thirty, and although no special precautions were taken in limiting the amount withdrawn, no inconvenience was suffered by the patients, who, as a rule, slept quietly for a few hours after being returned to bed. Manometric estimation of the pressure of the fluid was found to be unsatisfactory, as the patients were usually crying, and this rendered observations as to the rapidity of flow also unsatisfactory; but as far as

* Received for publication, May 14, 1913.

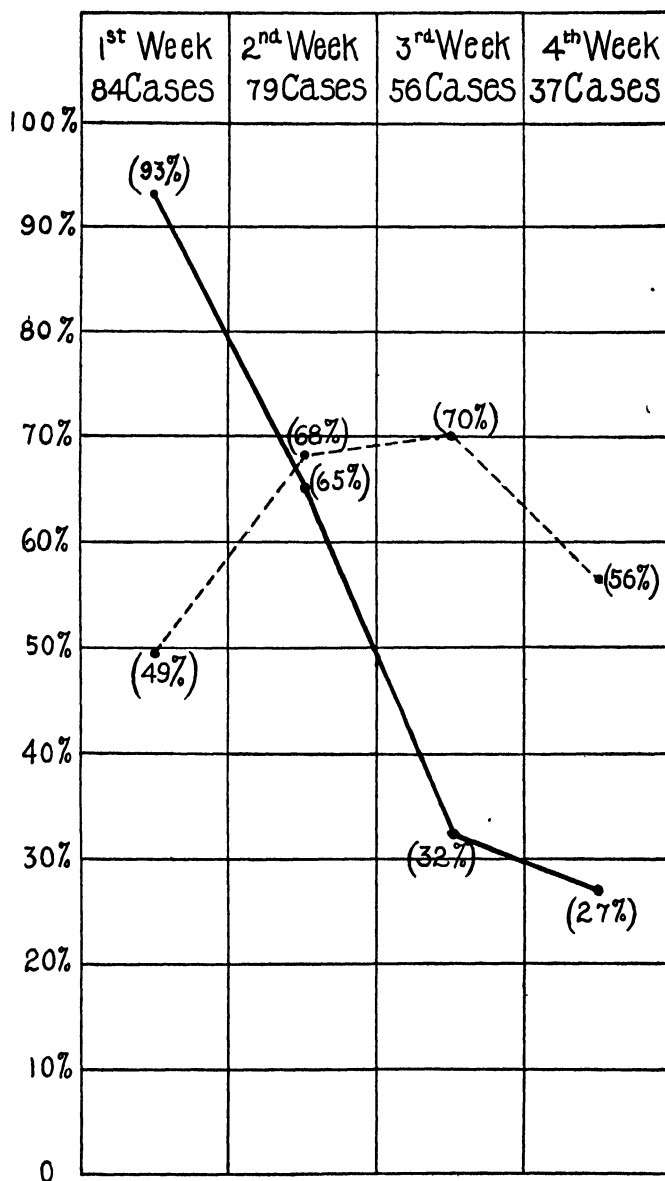
¹ Draper, G., and Peabody, F. W., *Am. Jour. Dis. Child.*, 1912, iii, 153.

could be judged from the rate of flow in the quieter or very ill patients, an especially high pressure was not usually present. In these patients a continuous stream lasted usually only a few seconds, following which the flow from the needle was in a steady stream of drops. The fluid was always examined immediately after withdrawal.

The following method of estimating the number of cells was employed: The fluid was mixed with acetic acid in a hematocytometer pipette for white blood corpuscles. 50 per cent. acetic acid was drawn up to the "1" mark on the stem and the pipette was then filled with the spinal fluid. After a thorough shaking two counting chambers were filled immediately and counts were made. The globulin was estimated by the butyric acid method of Noguchi, and the intensity of the reaction was noted as very slightly plus (v. s. +), denoting a faint cloud; slightly plus (s. +), a definite cloud settling to a fine precipitate; plus (+), a precipitate forming immediately; and double plus (+ +), a heavy flocculent precipitate. The reaction with Fehling's solution was tested by boiling one cubic centimeter of spinal fluid with one cubic centimeter of Fehling's solution and allowing the mixture to stand for ten or fifteen minutes. No quantitative estimation of the reducing power was attempted. Reduction took place with every fluid examined.

The macroscopic appearance of the fluids was constant; nearly all were clear and colorless, a few showed very slight opalescence, and a few contained small white flakes. Those containing the small white flakes were usually obtained in the later stages of the disease. On standing, a delicate clot formed in a very small number of cases, but no relation between this property and the globulin reaction, cell count, or severity of the disease could be made out.

Owing to the lack of precise knowledge of the character of the spinal fluid in normal children and in children suffering from other acute infections, it was necessary to adopt a somewhat arbitrary standard as to the changes to be regarded as pathological. A globulin reaction noted as slightly plus (s. +) or more has been considered abnormal, while all those noted very slightly plus (v. s. +) and negative (—) have been considered normal. Cell counts of over ten cells to the cubic millimeter have been considered as ab-



TEXT-FIG. I. Curves showing variation in cell count and in globulin content in the cerebrospinal fluid in acute poliomyelitis. — = per cent. of cases with cell count above normal; ---- = per cent. of cases with globulin content above normal.

normal, and those of ten or less as normal. With these standards it is improbable that a normal fluid has been classed as abnormal.

In text-figure 1 are shown two curves; the continuous line indicates the percentage of cases that showed abnormal cell counts in each week of the disease, while the interrupted line indicates the percentage of cases that showed abnormal globulin reactions in each week. The percentage was calculated from the number of cases examined in each week, but if the fluid from a given case was examined more than once during a week, only the highest figure was taken. From the curves it is seen that while the number of cells was highest early in the disease and gradually diminished later, the globulin reaction was at a maximum in the third week of the disease and then gradually diminished. Of the fluids from the eighty-four cases examined in the first week, seventy-eight, or 93 per cent., showed cell counts of over ten per cubic millimeter, and forty-one, or 49 per cent., showed a globulin reaction of slightly plus (s. +) or over; of the fluids from the seventy-nine cases examined in the second week fifty-one, or 65 per cent., showed abnormal cell counts, and fifty-four, or 68 per cent., showed abnormal globulin reactions; of the fluids from the fifty-six cases in the third week eighteen, or 32 per cent., gave abnormal cell counts, and thirty-nine, or 70 per cent., abnormal globulin reactions; of the fluids from the thirty-seven cases in the fourth week ten, or 27 per cent., gave abnormal cell counts, and twenty, or 54 per cent., abnormal globulin reactions. After the fourth week examinations were made only on cases showing persistent abnormalities, so that the figures for the later weeks have not been included; twenty-five cases were examined in the fifth week and twenty-four in the sixth week or later.

Of the eighty-four cases seen in the first week, in fifty the number of cells in the spinal fluid was fifty or more per cubic millimeter, while in six the number of cells was not increased. In one of the cases, seen on the seventh day of the disease and the third of paralysis, the spinal fluid contained but seven cells per cubic millimeter and gave a + globulin reaction. There was respiratory involvement and the patient died a few hours after the examination was made. Another one of the cases was classed among the abortive cases and the spinal fluid was never abnormal. In another the cell count was

never abnormal, but a ++ globulin reaction was present. In three other cases the spinal fluid later became abnormal. In one abortive case, seen on the second day of the disease, the number of cells in the spinal fluid was not increased, but on the following day the fluid contained sixty-two cells per cubic millimeter. Seventy-nine fluids from patients in the second week of the disease were examined, and in fourteen, or 18 per cent., the cells numbered fifty or more per cubic millimeter. Counts as high as this were found only once or twice among the fluids that were obtained in the later weeks. Of the fluids obtained during the third week, only 32 per cent. showed abnormal cell counts, while 70 per cent. showed abnormal globulin reactions. The most usual type of fluid examined was one with a moderate increase in the number of cells and a definite increase in the amount of globulin, but many fluids, especially in the earlier stages, showed a great increase of cells and only a slight increase of globulin that would be noted as s. + or v. s. +. Another quite distinct type is that of fluids containing less than ten cells per cubic millimeter and giving a globulin reaction of + or ++. The highest cell count noted was one of 1,221 per cubic millimeter on the second day of the paralysis; three other fluids contained over 500 cells per cubic millimeter, and twenty-seven contained between 500 and 100. Of these thirty-one fluids only twelve, or 39 per cent., gave a globulin reaction of + or over. Sixteen cases at no time showed an abnormal cell count and of these nine, or 56 per cent., showed a globulin estimation of + or ++, while in one fluid it was noted as +++. The globulin estimation has been of great value in cases seen for the first time late in the disease or in cases where the number of cells was never very high.

The increase in the number of cells was due in nearly every case to mononuclear cells. A comparison of the results obtained by examining the cells in the hematocytometer chamber and in a stained dry preparation showed that while the chamber method gave an approximate idea of the proportion of polymorphonuclear cells present, it was quite inefficient for differentiating the various kinds of mononuclear cells. The dry preparations were made by centrifuging a mixture of one part of spinal fluid and two parts of carefully centrifuged sterile serum, pouring off the supernatant fluid,

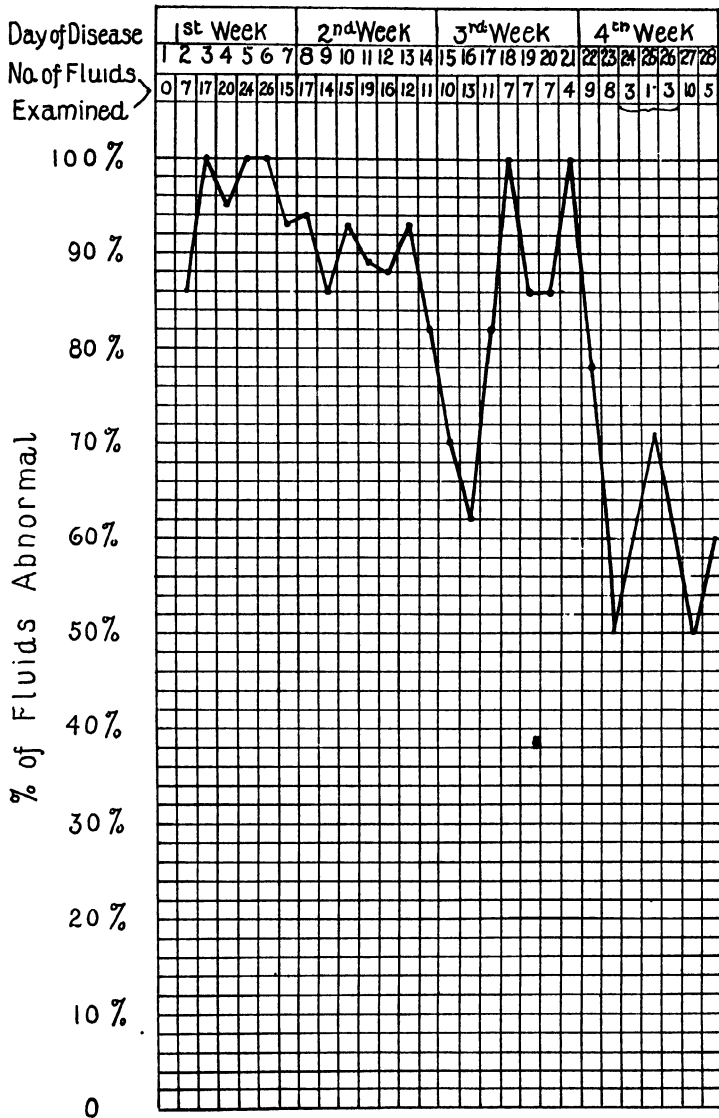
collecting the deposit in a fine capillary glass tube, and spreading it between two cover-glasses as in preparing a blood film. The staining method employed was similar to the May-Giemsa method of Pappenheim for blood films. The cover-glass preparations were fixed in Wright's or Hasting's solution for three minutes, and stained by the addition of an equal quantity of water for one minute. The fluid was then poured off and without washing the film was stained in Giemsa solution (1 to 15 dilution) for ten to fifteen minutes, the last stages of the process being watched under the low power of the microscope. By this method the cells were obtained well spread and in an apparently undamaged condition. The most common type of cell was the lymphocyte,—large, small, and intermediate. Almost all the cells in the fluid might be of this type. Large mononuclear cells with cytoplasm that was relatively more basophilic and a regular or an irregular or sometimes even a lobed nucleus were always present and might account for at least half the cells. Polymorphonuclear cells were usually absent, but were present in all the fluids obtained on the second day of the disease and in 80 per cent. of those obtained on the third day. The average differential count of these cells was 34 per cent. on the second day and 6 per cent. on the third. Endothelial cells like those seen in smears from the surface of the meninges were rare. In every fluid degenerating cells were found that showed no cytoplasm or only ragged shreds of cytoplasm and a nucleus that was breaking down. These cells were probably mononuclear in type, but classification was usually impossible. They were present in some fluids to the extent of 20 to 30 per cent. A few plasma cells could also be identified in some of the fluids. In the more acute stages large cells with very irregular nuclei and a faintly basophilic and vacuolated cytoplasm were present in considerable numbers. Large phagocytic cells were very rare. Accurate differential counts were not attempted in all cases, for an exact classification of the cells was difficult, every grade of transition from one type of mononuclear to another being found. In the examinations made, the following were the maximal and minimal proportions of each type:

Polymorphonuclear	0 to 85 per cent.
Lymphocytes (large, small, intermediate).....	17 to 89 per cent.
Endothelium of meninges	0 to 6 per cent.
Other types of mononuclear cells	3 to 70 per cent.
Degenerating cells	4 to 24 per cent.

The cases that showed many polymorphonuclear cells in the early stages later showed the usual mononuclear excess.

Lumbar puncture was performed in ten cases that were either in the preparalytic stage or that never developed definite parietic symptoms. The fluids from two children seen on the second day after onset of symptoms contained 880 and 113 cells per cubic millimeter, and they developed typical paralyses one and two days later, respectively. The fluid from one case seen on the fourth day of symptoms contained 650 cells per cubic millimeter and gave a + globulin reaction. The child developed respiratory paralysis three days later and died. Another case seen on the sixth day, with a fluid containing thirty-seven cells per cubic millimeter, developed paralysis two days later. The spinal fluids of six abortive cases were examined and five of them contained an increase in the number of cells, the number varying from thirty-four to eighty-nine per cubic millimeter. The sixth abortive case was that of a child who suffered from fever and irritability, with tenderness and slight stiffness of the neck, and who was living in the same house with a child who, a few days later, developed typical paralysis. Lumbar puncture was performed on two occasions, the fourth and tenth days after onset of symptoms, but on both occasions the fluid showed no abnormalities. From one of the abortive cases a normal fluid was obtained on the second day after onset of the symptoms, and a fluid containing sixty-two cells per cubic millimeter on the following day. The large proportion of cases that gave abnormal fluids early in the disease is represented in text-figure 2, where the number of fluids abnormal in either cell count, globulin content, or both, examined on each day of the disease, is represented.

From this figure it is seen that nearly every fluid obtained during the first week after onset of symptoms was abnormal. During the second week the number of abnormal fluids diminished slightly, and at the beginning of the third week the percentage dropped to 62



TEXT-FIG. 2. Curve showing per cent. of cases with abnormal cerebrospinal fluids, on each day of the disease in acute poliomyelitis.

per cent. In the second half of the third week the percentage rose again. This rise was due to the number of fluids giving abnormal globulin reactions at this period of the disease. After the fourth week so few fluids were examined on any one day that the daily figures are of little value. From the point of view of estimating the value of lumbar puncture in making an early diagnosis, it is important to know on what day after the onset of symptoms the first signs of paralysis were noted. It was found that paralysis was noted on the same day as the first definite symptom; that is, on the first day of the disease in five cases, on the second day in thirty cases, on the third in thirty-two cases, on the fourth in sixteen cases, on the fifth in fourteen cases, on the sixth in seven cases, on the seventh in two cases, on the eighth in four cases, on the tenth in one case, and on the fifteenth in one case. In most cases, then, paralysis developed after two or more days of preparalytic symptoms. Of the spinal fluids examined on the second day after onset of symptoms, 86 per cent. were abnormal, and all of those examined on the third day showed definite abnormalities. It would appear, therefore, that in most cases the spinal fluid would give indications of pathological conditions before the onset of paralysis. In tuberculous meningitis and in syphilitic myelitis the clinical picture closely resembles that of acute epidemic poliomyelitis, and the characters of the spinal fluid are similar as regards the number and type of cells present and the character of the globulin reaction. The finding of the tubercle bacillus in the fluid in tuberculous meningitis and a positive Wassermann reaction in the spinal fluid in syphilitic myelitis is a sufficient differentiation, as the Wassermann reaction was performed on the fluids of all the poliomyelitis cases in the hospital in 1912 and was negative in every case.

The relation between the severity of the attack and the changes in the spinal fluid is not capable of accurate determination. While all the cases that died during the acute stages showed marked abnormalities in the number of cells present or in the globulin reaction, comparatively mild or even abortive cases were accompanied by just as marked abnormalities. Nor did the cases that showed the more extensive changes in their spinal fluids always give clinical

signs of more extensive meningeal involvement. The localization of the paralysis did not seem to affect the extent of the changes in the fluids; the cases that showed involvement of the lower limbs and thus of the lower segments of the spinal cord did not consistently show greater changes in the spinal fluid obtained by lumbar puncture, while those cases that showed clinical evidence of lesions confined to the upper part of the cord or to the bulb often showed as great changes in the fluid.

The prognosis as to life depends on the localization of the paralysis as well as on the severity, while the prognosis as to ultimate return of muscular power depends rather on the completeness of the damage done to any particular part of the spinal cord. The examination of the spinal fluid does not seem to offer evidence as to the localization or the severity of the damage.

CONCLUSIONS.

The spinal fluid in the cases of acute epidemic poliomyelitis examined was usually clear, colorless, and did not appear to be under any great increase of pressure. It showed changes in the number of cells present, or in the globulin content, or in both, in the majority of cases examined on the first few days after onset of symptoms. The number of cells was usually highest during the first week, and in one case reached the figure of 1,221 per cubic millimeter. The globulin reaction was usually most marked during the third week. The number of cells diminished rapidly and was above normal in only 32 per cent. of the cases in the third week. The increase in the globulin reaction persisted to the fourth week and might be present for a considerably longer period. The cell increase was due almost invariably to mononuclear cells of various types. The lymphocytic type of cell was the most common. A high polymorphonuclear count was noted in the very early stages. All the fluids reduced Fehling's solution. The examination of the spinal fluid may be of value in diagnosis in the preparalytic stages and in abortive cases. It is not of value in prognosis as to life or ultimate recovery.

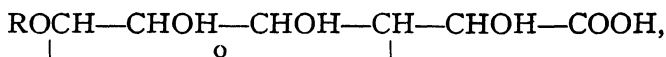
ON THE EXCRETION OF GLYCURONIC ACID IN PNEU- MOCOCCUS INFECTIONS, WITH SPECIAL REFER- ENCE TO LOBAR PNEUMONIA.*

By FLORENTIN MEDIGRECEANU, M.D.

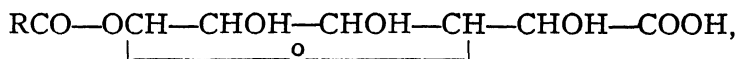
(From the Hospital of The Rockefeller Institute for Medical Research, New
York.)

The present problem was suggested by the fact that glycuronic acid has the property of combining in the living animal organism with a great many chemically well defined organic substances, which may be either products occurring under normal conditions in the animal body, or drugs or other chemicals foreign to the animal organism.¹ They belong to almost all chemical groups. It is generally admitted that after the reaction has taken place in the organism and the double compounds have been formed, they are excreted in the urine without further transformation.

There are two types of chemical combination that have been recognized in these compounds: (1) the glucosidic type,



demonstrated for the first time by Fisher and Piloty,² and (2) the ester type,



discovered by Jaffé.³ It is thus obvious that the chemical functions that react with the aldehyde group of glycuronic acid are alcohol, phenol, and acid. The substances that do not possess these chemical groups have to undergo certain changes before they become suitable for reaction.

* Received for publication, May 19, 1913.

¹ A complete review of the subject is given by Neuberg, C., *Der Harn, sowie die übrigen Ausscheidungen und Körperflüssigkeiten*, Berlin, 1911, i, 429.

² Fisher, E., and Piloty, O., *Ber. d. deutsch. chem. Gesellsch.*, 1891, xxiv, 522.

³ Jaffé, M., *Ztschr. f. physiol. Chem.*, 1904-5, xliii, 374.

Since a great many of the substances that are excreted in the urine, combined with glycuronic acid, have a marked toxic effect on the animal organism, this acid is generally regarded as a detoxifying agent, as are also sulphuric acid and glycocoll. Mayer and Neuberg found that glycuronic acid is a constant ingredient of the normal urine of man and other mammals.⁴ This acid is always excreted in a conjugated form, and, according to these authors, the conjugated bodies are phenol and indoxyl.

The present paper deals with the excretion in the urine of glycuronic acid during pneumococcus lobar pneumonia, and a few other pulmonary or pleural febrile affections. Its excretion by rabbits with general pneumococcus infections has also been studied.⁵ Quantitative relations merely are considered. No attempt has been made to estimate and identify the conjugated bodies.

METHOD.

All estimations have been made according to the method of B. Tollens and his co-workers,⁶ as modified for urine by C. Tollens.⁷ This method gives better results than the ones formerly used (the p-bromphenylhydrazine and the polarimetric methods). It is based on the fact that glycuronic acid, in a free as well as in a conjugated state, yields furfural by boiling with hydrochloric acid. The furfural may be distilled over with the hydrochloric acid and precipitated with phloroglucin. The amount of furfural-phloroglucin precipitate multiplied by three represents the original quantity of glycuronic acid.

The directions given by Tollens have been followed with but slight modifications. 250 cubic centimeters of fresh urine (the twenty-four hour specimen of a rabbit) are first precipitated

⁴ Mayer, P., and Neuberg, C., *Ztschr. f. physiol. Chem.*, 1900, xxix, 256.

⁵ The excretion of glycuronic acid in disease (chiefly in diabetes and dyspneic conditions) has been studied by various investigators (Neuberg, *loc. cit.*). Their figures, however, cannot be used for comparison with those obtained in this work, as they used the polarimetric method of estimation, and it was found that the results do not coincide.

⁶ Lefevre, K. U., and Tollens, B., *Ber. d. deutsch. chem. Gesellsch.*, 1907, xl, 4513.

⁷ Tollens, C., *Ztschr. f. physiol. Chem.*, 1909, lxi, 95; 1910, lxvii, 138; Tollens, C., and Stern, F., *ibid.*, 1910, lxiv, 39.

with five cubic centimeters of ammonia and with basic lead acetate solution until all precipitable substances are thrown down. An excess of reagent must be carefully avoided. After standing for at least sixteen hours, the precipitate is separated from the liquid by centrifugalization and thoroughly washed three times with distilled water in 100 cubic centimeter tubes.⁸ If necessary, a few drops of ammonia are added to each washing to prevent the solution of the precipitate. The precipitate is then brought quantitatively into a flask of one liter capacity, 12 per cent. hydrochloric acid being used for rinsing the tubes, and subjected to distillation in an apparatus similar to the arrangement used for distilling ammonia in the Kjehldahl process. The flask contains first about 120 cubic centimeters of precipitate and hydrochloric acid. After distilling over sixty cubic centimeters of solution, a fresh amount of sixty cubic centimeters of 12 per cent. hydrochloric acid is added to the flask and the same operation repeated until 600 cubic centimeters of distillate are obtained.

The distilled solution is then filtered and 0.25 of a gram of phloroglucin dissolved in 12 per cent. hydrochloric acid is added. In order to determine whether or not all the amount of furfurol has distilled over, about 100 cubic centimeters more of distillate are collected in a separate beaker and the amount of furfurol present is tested by adding a little phloroglucin solution.⁹ The distillation is continued until phloroglucin no longer precipitates. An amount of 600 cubic centimeters of distillate, however, has been found sufficient in almost all cases. The furfurol-phloroglucid is allowed to stand for at least sixteen hours, is then collected on a Gooch filter, thoroughly washed with 150 cubic centimeters of distilled water, and dried to constant weight at 100° C., the crucibles being kept in closed, glass stoppered weighing bottles, in order to avoid the rapid absorption of moisture by the precipitate. In calculating the total amount of furfurol-phloroglucid, a correction for the quantity remaining in solution

⁸ No filter paper is used. The usual correction for the amount of furfurol developed by filter paper when boiling with hydrochloric acid thus becomes superfluous.

⁹ This procedure was preferred to the color test with anilin acetate used by Tollens.

must be made. This is 0.0092 of a gram per liter of distillate, as given by Tollens.

About sixty estimations have been made on normal individuals and on non-febrile syphilitic patients, without gastro-intestinal disturbances. According to Tollens, a normal adult on a mixed diet usually excretes 0.3 to 0.4 of a gram of glycuronic acid in twenty-four hours. The figures are slightly higher when the diet is rich in meat and fat. Occasionally the excretion may be much larger (up to 0.82 of a gram) without giving any evidence of a pathological process.

The findings of Tollens have been generally confirmed. The variations, however, from day to day and with the change in diet have been more frequent and larger than in the cases of Tollens. Normal persons and non-febrile syphilitic patients weighing fifty-five to seventy kilograms, on a mixed diet composed of milk, bread, vegetables, fruits, and moderate amounts of meat, excreted daily 0.4 to 0.6 of a gram of glycuronic acid. Not infrequently, however, with a large intake, the quantities of glycuronic acid excreted were as high as 0.8 and even 0.95 of a gram. For instance, H. B. (table III), a white male patient convalescing from pneumonia, weighing fifty-five kilograms, excreted daily 0.9 and 0.93 of a gram on a rich diet of milk, soup, bread, butter, cocoa, coffee, steak, roast beef, spinach, cereals, string beans, rice pudding, eggs, and prunes. Eight days later the average output of the same patient was only 0.6 of a gram of glycuronic acid, the diet being composed of milk, bread, butter, and small amounts of chicken and beef.

The results have been cited in order to show that in judging the significance of the quantity of glycuronic acid excreted in the cases that follow (tables I to XIII), the quality as well as the quantity of the intake must be considered. The analyses are from patients who did not show an appreciable gastro-intestinal disturbance during the disease. The urine contained only traces of albumin even during the high febrile period, and in no phase of the disease was there present a demonstrable amount of sugar (tested by the reduction of Fehling's solution).

TABLE I.

*R. F., White Male Patient, Age Thirty-Five Years, Weight Seventy Kilograms.
 Diagnosis: Pneumonia, Lobar; Consolidation of Left Lower Lobe; Transitory
 Septicemia and Pleurisy, Serofibrinous.*

Date.	Day of disease.	Urine in c.c. Specific gravity at 15°C.	Furfural-glucid in gm.	Glucuron in gm.	Rectal temperature, C. Maximum. Minimum.	Intake and remarks.
1912 Nov. 18	6	$\frac{1.350}{1.023}$	0.404	1.212	41.5° 37.3°	<i>Drugs:</i> codein 64 mg., morphia 11 mg. <i>Food:</i> water 2,800 c.c., milk 1,050 c.c., orange juice 340 c.c., albumen 80 c.c., cream soup 150 c.c., eggs 1, rice 30 gm. <i>White blood cells:</i> 16,000.
Nov. 19	7	$\frac{1.380}{1.025}$	0.392	1.176	41.3° 37.0°	<i>Drugs:</i> codein 64 mg., morphia 11 mg. <i>Food:</i> water 3,000 c.c., milk 1,300 c.c., soup 200 c.c., cereal 30 gm., eggs 2, potatoes 30 gm., rice 45 gm., toast 1 piece.
Nov. 20	8	$\frac{1.340}{1.019}$	0.324	0.972	38.0° 37.0°	<i>Drugs:</i> none. <i>Food:</i> water 2,700 c.c., milk 1,100 c.c., orange juice 70 c.c., cream soup 200 c.c., albumen 30 c.c., cereal 75 gm., eggs 3, rice 30 gm., toast 3 pieces, tapioca pudding 45 gm., sugar 10 gm., potatoes 45 gm.
Nov. 21	9	$\frac{1.610}{1.018}$	0.340	1.020	37.5° 37.0°	<i>Drugs:</i> none. <i>Food:</i> water 1,500 c.c., milk 1,100 c.c., orange juice 35 c.c., cream soup 100 c.c., cereal 75 gm., eggs 2, toast 3 pieces, potatoes 45 gm., albumen 25 c.c., rice 45 gm.
Nov. 22	10	$\frac{1.490}{1.023}$	0.325	0.975	37.5° 37.3°	<i>Drugs:</i> codein 32 mg. <i>Food:</i> water 2,200 c.c., milk 1,000 c.c., orange juice 160 c.c., cream soup 150 c.c., albumen 30 c.c., cereal 75 gm., eggs 2, toast 2 pieces, potatoes 30 gm., sugar 20 gm.
Nov. 23	11	$\frac{1.360}{1.020}$	0.335	1.005	37.6° 37.0°	<i>Drugs:</i> codein 32 mg. <i>Food:</i> water 1,500 c.c., milk 1,200 c.c., orange juice 220 c.c., albumen 50 c.c., cereal 60 gm., eggs 2, toast 3 pieces, custard 45 gm., potatoes 35 gm., crackers 2 pieces, baked apple 1.
Nov. 24	12	$\frac{1.410}{1.023}$	0.288	0.864	37.5° 37.2°	<i>Drugs:</i> codein 32 mg. <i>Food:</i> water 1,000 c.c., milk 1,400 c.c., orange juice 220 c.c., albumen 70 c.c., cereal 130 gm., eggs 2, toast 5 pieces, soup 250 c.c., potatoes 45 gm., spinach 30 gm., sugar 20 gm., pudding 30 gm.
Nov. 25	13	$\frac{940}{1.026}$	0.237	0.711	37.5° 36.8°	<i>Drugs:</i> codein 32 mg. <i>Food:</i> water 1,300 c.c., milk 1,000 c.c., orange juice 250 c.c., albumen 25 c.c., soup 150 c.c., cream 50 c.c., ice cream 45 gm., eggs 4, toast 3 pieces, pudding 30 gm., cereal 45 gm.

TABLE I.—*Concluded.*

Date.	Day of disease.	Urine in c.c. Specific gravity at 15° C.	Furfural-glucid in gm.	Glucose in gm.	Rectal temperature, C. Maximum. Minimum.	Intake and remarks.
1912 Nov. 26	14	$\frac{1,100}{1,028}$	0.266	0.798	37.5° 37.0°	<i>Drugs:</i> codein 32 mg. <i>Food:</i> water 1,500 c.c., milk 1,000 c.c., orange juice 250 c.c., albumen 25 c.c., soup 250 c.c., cream 50 c.c., rice 75 gm., potatoes 15 gm., bread 4 slices, pudding 15 gm., eggs 3, cereal 30 gm.
Nov. 27	15	$\frac{950}{1,027}$	0.227	0.681	37.5° 36.7°	<i>Drugs:</i> none. <i>Food:</i> water 1,800 c.c., milk 1,000 c.c., orange juice 250 c.c., albumen 25 c.c., soup 250 c.c., creamed celery 60 gm., toast 7 pieces, cocoa 250 c.c., eggs 4, cereal 60 gm.
Dec. 13	31	$\frac{1,150}{1,025}$	0.284	0.852	37.5° 37.0°	<i>Drugs:</i> none. <i>Food:</i> copious mixed diet with meat and fruits.
Dec. 14	32	$\frac{1,400}{1,027}$	0.279	0.837	37.4° 36.6°	<i>Drugs:</i> none. <i>Food:</i> copious mixed diet with meat and fruits. Patient recovered and was discharged 5 days later.

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TABLE II.

*M. B., White Female Patient, Age Eighteen Years, Weight Forty-Five Kilograms.
Diagnosis: Pneumonia, Lobar; Involvement of Right Upper Lobe.*

Date.	Day of disease.	Urine in c.c. Specific gravity at 15°C.	Furfural-phloroglucin in gm.	Glucuron in gm.	Rectal temperature, C. Maximum. Minimum.	Intake and remarks.
1912 Nov. 19	4	$\frac{1,570}{1,012}$	0.353	1.059	39.5° 38.7°	<i>Drugs:</i> none. <i>Food:</i> water 1,300 c.c., milk 520 c.c., orange juice 310 c.c., lemon juice 12 c.c., albumen 120 c.c., sugar 20 gm.
Nov. 20	5	$\frac{1,630}{1,019}$	0.295	0.885	39.9° 39.3°	<i>White blood cells:</i> 46,000. <i>Drugs:</i> codein 96 mg. <i>Food:</i> water 1,700 c.c., milk 750 c.c., orange juice 250 c.c., milk soup 150 c.c., albumen 85 c.c.
Nov. 21	6	$\frac{1,390}{1,012}$	0.314	0.942	39.9° 39.0°	<i>Drugs:</i> morphia 24 mg. <i>Food:</i> water 1,600 c.c., milk 900 c.c., grape juice 100 c.c., orange juice 40 c.c., albumen 60 c.c., sugar 10 gm.
Nov. 22	7	$\frac{1,870}{1,019}$	0.279	0.837	39.9° 38.5°	<i>Drugs:</i> codein 96 mg. <i>Food:</i> water 1,400 c.c., milk 1,500 c.c., orange juice 200 c.c., albumen 70 c.c., sugar 15 gm.
Nov. 23	8	$\frac{2,130}{1,009}$	0.351	1.053	38.5° 37.3°	<i>Drugs:</i> codein 64 mg. <i>Food:</i> water 1,400 c.c., milk 800 c.c., orange juice 270 c.c., albumen 75 c.c., sugar 20 gm.
Nov. 24	9	$\frac{1,080}{1,017}$	0.251	0.753	37.4° 37.3°	<i>Drugs:</i> none. <i>Food:</i> water 1,000 c.c., milk 850 c.c., orange juice 270 c.c., albumen 75 c.c., sugar 20 gm.
Nov. 25	10	$\frac{830}{1,022}$	0.244	0.732	37.7° 37.3°	<i>Drugs:</i> none. <i>Food:</i> water 700 c.c., milk 860 c.c., orange juice 210 c.c., albumen 45 c.c., eggs 1, ice cream 75 gm., rice 60 gm., tapioca 45 gm., toast 3 pieces.
Nov. 26	11	$\frac{1,380}{1,016}$	0.279	0.837	37.3° 37.0°	<i>Drugs:</i> none. <i>Food:</i> water 700 c.c., milk 300 c.c., orange juice 110 c.c., cream soup 200 c.c., albumen 25 c.c., cocoa 150 c.c., bread 1 slice, cereal 75 gm., eggs 1, toast 2 pieces, potatoes 30 gm.
Nov. 27	12	$\frac{1,150}{1,020}$	0.230	0.690	37.2° 36.5°	<i>Drugs:</i> none. <i>Food:</i> water 900 c.c., milk 800 c.c., cocoa 250 c.c., orange juice 200 c.c., cream soup 200 c.c., cereal 60 gm., albumen 25 c.c., eggs 1, bread 7 slices, creamed celery 20 gm., rice custard 45 gm., pudding 30 gm.
Dec. 4	19	$\frac{900}{1,026}$	0.289	0.867	37.1° 36.3°	<i>Drugs:</i> none. <i>Food:</i> copious mixed diet, meat and fruits included.
Dec. 5	20	$\frac{1,010}{1,022}$	0.249	0.747	37.3° 36.5°	<i>Drugs:</i> none. <i>Food:</i> copious mixed diet, meat and fruits included. Patient recovered. She was discharged 4 days later.

TABLE III.

H. B., White Male Patient, Age Twenty-Two Years, Weight Fifty-Five Kilograms.

Diagnosis: Pneumonia, Lobar (Relapse); Involvement of Right Upper and Lower Lobes.

Date.	Day of disease.	Urine in c.c. Specific gravity at 15° C.	Furfural-phloroglucid in gm.	Glucuron in gm.	Rectal temperature, C. Maximum. Minimum.	Intake and remarks.
1912 Dec. 9	16	$\frac{2,080}{1,014}$	0.400	1.200	40.5° 39.6°	<i>Drugs:</i> codein 32 mg., morphia 16 mg. <i>Food:</i> lactose 360 gm., water 4,000 c.c., milk 950 c.c., orange juice 700 c.c., lemon juice 100 c.c., eggs 1, albumen 50 c.c.
Dec. 10	17	$\frac{1,470}{1,017}$	0.435	1.305	40.3° 39.6°	<i>Drugs:</i> codein 32 mg., morphia 16 mg. <i>Food:</i> lactose 420 gm., water 2,600 c.c., milk 130 c.c., orange juice 550 c.c., cocoa 150 c.c., cream soup 250 c.c., albumen 20 c.c.
Dec. 11	18	$\frac{3,350}{—}$	0.670	2.010	40.3° 40.0°	<i>Drugs:</i> morphia 48 mg., caffein sodium salicylate 160 mg., camphor oil 400 mg. <i>Food:</i> lactose 360 gm., water 2,300 c.c., milk 1,900 c.c., orange juice 450 c.c., albumen 10 c.c., cream soup 200 c.c.
Dec. 12	19	$\frac{1,770}{1,015}$	0.457	1.371	40.0° 38.5°	<i>Drugs:</i> morphia 32 mg., caffein sodium salicylate 224 mg., camphor oil 500 mg. <i>Food:</i> lactose 300 gm., water 2,300 c.c., milk 1,400 c.c., orange juice 350 c.c., cocoa 750 c.c., lemon juice 20 c.c., albumen 30 c.c.
Dec. 13	20	$\frac{1,590}{1,015}$	0.468	1.404	38.5° 36.8°	<i>Drugs:</i> codein 32 mg., caffein sodium salicylate 160 mg., camphor oil 100 mg. <i>Food:</i> lactose 200 gm., water 1,400 c.c., milk 1,400 c.c., orange juice 550 c.c., cocoa 400 c.c., custard 30 gm., albumen 30 c.c.
Dec. 14	21	$\frac{970}{1,024}$	0.351	1.053	37.5° 36.2°	<i>Drugs:</i> caffein sodium salicylate 32 mg., morphia 16 mg. <i>Food:</i> lactose 40 gm., water 1,400 c.c., milk 1,000 c.c., orange juice 450 c.c., coffee 100 c.c., cocoa 200 c.c., eggs 1, albumen 30 c.c., custard 30 gm., bread 1 piece.
Dec. 31	38	$\frac{940}{1,021}$	0.302	0.906	37.2° 36.8°	<i>Food:</i> copious mixed diet,—milk, soup, cocoa, coffee, meat, vegetables, fruits, eggs.
1913 Jan. 1	39	$\frac{1,180}{1,024}$	0.312	0.936	37.3° 36.8°	<i>Food:</i> copious mixed diet,—milk, soup, cocoa, coffee, meat, vegetables, fruits, eggs.
Jan. 9-10	47-48	$\frac{1,080}{1,028}$	0.200	0.600	37.0° 36.5°	Average amounts of two days. <i>Food:</i> moderate quantity of milk, bread, chicken, and beef. Patient recovered. He was discharged on Jan. 10.

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TABLE IV.

C. F., Colored Male Patient, Age Thirty-Seven Years, Weight Sixty-Three Kilograms.

Diagnosis: Pneumonia, Lobar; Consolidation of Right Lower Lobe.

Date.	Day of disease.	Urine in c.c. Specific gravity at 15°C.	Furfurophloroglucid in gm.	Glucuron in gm.	Rectal temperature, C. Maximum. Minimum.	Intake and remarks.
1912 Dec. 11	5	$\frac{780}{1,025}$	0.254	0.762	40.5° 39.3°	<i>Drugs:</i> codein 32 mg. <i>Food:</i> lactose 440 gm., water 3,300 c.c., milk 1,800 c.c., orange juice 75 c.c.
Dec. 12	6	$\frac{1,190}{1,021}$	0.302	0.906	40.0° 37.3°	<i>Drugs:</i> none. <i>Food:</i> lactose 400 gm., water 4,600 c.c., milk 1,800 c.c., orange juice 300 c.c., cocoa 150 c.c.
Dec. 13	7	$\frac{1,100}{1,018}$	0.219	0.657	37.3° 36.8°	<i>Drugs:</i> codein 32 mg. <i>Food:</i> lactose 240 gm., water 2,800 c.c., milk 1,000 c.c., orange juice 100 c.c., soup 150 c.c., albumen 20 c.c., custard 30 gm., bread 1 slice.
Dec. 14	8	$\frac{1,270}{1,016}$	0.262	0.786	37.5° 37.0°	<i>Drugs:</i> none. <i>Food:</i> water 1,700 c.c., milk 1,000 c.c., orange juice 200 c.c., custard 45 gm., albumen 30 c.c., bread 2 slices.
1913 Jan. 8	33	$\frac{790}{1,027}$	0.208	0.624	37.5° 36.7°	<i>Food:</i> mixed diet,—milk, meat, bread, small amounts of vegetables.
Jan. 9	34	$\frac{1,010}{1,022}$	0.193	0.579	37.0° 36.3°	<i>Food:</i> mixed diet,—milk, meat, bread, small amounts of vegetables. Patient recovered. He was discharged 2 days later.

TABLE V.

F. R., White Female Patient, Age Twenty-Nine Years, Weight Forty-Five Kilograms.

Diagnosis: Pneumonia, Lobar; Involvement of Right Lower Lobe.

Date.	Day of disease.	Urine in c.c. Specific gravity at 15° C.	Furfurophloroglucid in gm.	Glucuron in gm.	Rectal temperature. C. Maximum. Minimum.	Intake and remarks.
1912 Dec. 11	8	$\frac{1,750}{1,010}$	0.291	0.873	40.3° 38.0°	<i>Drugs:</i> codein 32 mg. <i>Food:</i> water 1,600 c.c., milk 550 c.c., orange juice 170 c.c., cocoa 200 c.c., bread 1 slice, custard 30 gm. <i>White blood cells:</i> 16,000.
Dec. 12	9	$\frac{1,410}{1,012}$	0.242	0.726	38.0° 37.0°	<i>Drugs:</i> none. <i>Food:</i> water 1,000 c.c., milk 550 c.c., tea 200 c.c., coffee 200 c.c., cocoa 300 c.c., orange juice 150 c.c., toast 1 piece, eggs 2, prunes 4, sugar 10 gm.
Dec. 13	10	$\frac{910}{1,016}$	0.228	0.684	37.0° 36.7°	<i>Drugs:</i> none. <i>Food:</i> water 1,000 c.c., milk 450 c.c., orange juice 50 c.c., soup 200 c.c., cocoa 150 c.c., tea 150 c.c., eggs 2, pudding 90 gm., bread and toast 3 pieces, chicken 50 gm.
Dec. 20	17	$\frac{1,360}{1,017}$	0.230	0.690	36.7° 36.5°	<i>Food:</i> copious mixed diet, including meat and fruits.
Dec. 21	18	$\frac{1,010}{1,019}$	0.248	0.744	36.7°	<i>Food:</i> copious mixed diet, including meat and fruits. Patient recovered. She was discharged on Dec. 28.

TABLE VI.

*A. H., Colored Male Patient, Age Six Years, Weight Twenty Kilograms.**Diagnosis: Pneumonia, Lobar; Involvement of Left Lower Lobe.*

Date.	Day of disease	Urine in c.c. Specific gravity at 15°C.	Furfural-phenol-glucid in gm.	Glucuron in gm.	Rectal temperature, C. Maximum. Minimum.	Intake and remarks.
1912 Dec. 21	5	<u>750</u> 1.013	0.122	0.366	40.0° 39.4°	<i>Drugs:</i> none. <i>Food:</i> water 1,300 c.c., milk 250 c.c., orange juice 160 c.c., albumen 50 c.c., cocoa 150 c.c., sugar 10 gm. <i>White blood cells:</i> 23,000.
Dec. 22	6	<u>870</u> 1.009	0.120	0.360	39.6° 37.0°	<i>Drugs:</i> none. <i>Food:</i> water 700 c.c., milk 300 c.c., orange juice 270 c.c., albumen 50 c.c., cocoa 350 c.c., lemon juice 20 c.c.
Dec. 23	7	<u>390</u> 1.010	0.070	0.210	37.5° 37.2°	<i>Drugs:</i> castor oil 15 c.c. <i>Food:</i> water 600 c.c., milk 770 c.c., orange juice 260 c.c., albumen 50 c.c., rice 45 gm., bread 4 slices, toast 3 slices, custard 45 gm., lemon juice 50 c.c.
Dec. 24	8	<u>950</u> 1.012	0.146	0.438	37.3° 37.2°	<i>Drugs:</i> codein 10 mg. <i>Food:</i> Water 860 c.c., milk 800 c.c., orange juice 150 c.c., bread 4 slices, soup 200 c.c., rice 30 gms., cocoa 200 c.c., eggs 2.
Dec. 25	9	<u>1,150</u> 1.014	0.163	0.489	37.4° 37.0°	<i>Drugs:</i> none. <i>Food:</i> water 800 c.c., milk 850 c.c., prunes 8, orange juice 100 c.c., soup 200 c.c., bread 4 slices, rice 15 gm., coffee jelly 30 gm., eggs 2, albumen 20 c.c.
Dec. 31	15	<u>980</u> 1.014	0.195	0.585	37.7° 37.0°	<i>Food:</i> copious mixed diet, including meat and fruits.
1913 Jan. 1	16	<u>1,300</u> 1.014	0.192	0.576	37.7° 37.4°	<i>Food:</i> copious mixed diet, including meat and fruits.
Jan. 8	23	<u>990</u> 1.017	0.116	0.348	37.7° 37.2°	<i>Food:</i> mixed diet,—moderate amounts of fruits and vegetables.
Jan. 9	24	<u>1,130</u> 1.013	0.115	0.345	37.6° 37.4°	<i>Food:</i> mixed diet,—moderate amounts of fruits and vegetables. Patient recovered. He was discharged on Jan. 10.

TABLE VII.

M. M., White Male Patient, Age Twenty-Three Years, Weight Fifty-Four Kilograms.

Diagnosis: Pneumonia, Lobar; Involvement of Right Upper and Lower Lobes.

Date.	Day of disease.	Urine in c.c. Specific gravity at 15° C.	Furfurophloroglucid in gm.	Glucuron in gm.	Rectal temperature, C. Maximum. Minimum.	Intake and remarks
1912 Dec. 18	4	$\frac{1.020}{1.024}$	0.194	0.582	40.5° 40.0°	<i>Drugs:</i> morphia 11 mg., codein 10 mg., calomel 64 mg., magnesium sulphate 15 gm. <i>Food:</i> water 1,200 c.c., milk 700 c.c., broth 150 c.c., orange juice 200 c.c., cocoa 150 c.c., albumen (egg) 20 c.c. <i>White blood cells:</i> 28,000.
Dec. 19	5	$\frac{1.310}{1.023}$	0.261	0.783	40.4° 39.6°	<i>Drugs:</i> codein 16 mg. <i>Food:</i> water 2,000 c.c., milk 810 c.c., broth 350 c.c., cocoa 300 c.c.
Dec. 20	6	$\frac{1.480}{1.023}$	0.233	0.699	39.6° 38.8°	<i>Drugs:</i> codein 16 mg. <i>Food:</i> water 1,500 c.c., milk 700 c.c., soup 200 c.c., cocoa 350 c.c.
Dec. 21	7	$\frac{1.030}{1.025}$	0.207	0.621	38.8° 37.7°	<i>Drugs:</i> codein 24 mg., morphia 11 mg. <i>Food:</i> water 1,300 c.c., milk 450 c.c., orange juice 50 c.c., albumen 20 c.c., sugar 10 gm., toast 2 slices.
Dec. 22	8	$\frac{950}{1.026}$	0.201	0.603	37.8° 37.2°	<i>Drugs:</i> codein 16 mg., morphia 11 mg. <i>Food:</i> water 700 c.c., milk 500 c.c., cocoa 300 c.c., orange juice 80 c.c., eggs 2, albumen 20 c.c., bread and toast, 4 slices.
Dec. 23	9	$\frac{1.160}{1.024}$	0.237	0.712	38.7° 37.8°	<i>Drugs:</i> codein 32 mg. <i>Food:</i> water 1,200 c.c., milk 550 c.c., soup 200 c.c., orange juice 150 c.c., cocoa 300 c.c., custard 25 gm., eggs 2, toast 2 pieces. Patient recovered. He was discharged on Jan. 16.

TABLE VIII.

*N. S., White Male Patient, Age Twenty-Two Years, Weight Sixty Kilograms.
Diagnosis: Pneumonia, Lobar; Consolidation of Right Lower Lobe; Empyema.*

Date.	Day of disease.	Urine in c.c. Specific gravity at 15°C.	Furfurophloroglucid in gm.	Glucuron in gm.	Rectal temperature, C Maximum. Minimum.	Intake and remarks.
1912 Dec. 8	10	$\frac{1,820}{1,016}$	0.375	1.025	40.5° 39.5°	<i>Drugs:</i> codein 96 mg. <i>Food:</i> water 2,000 c.c., milk 300 c.c., orange juice 250 c.c., lemon juice 100 c.c., sugar 30 gm. <i>White blood cells:</i> 20,000.
Dec. 9	11	$\frac{1,880}{1,017}$	0.435	1.305	39.9° 39.0°	<i>Drugs:</i> codein 32 mg. <i>Food:</i> water 2,000 c.c., lemon juice 170 c.c., albumen 70 c.c., tomato soup 70 c.c.
Dec. 10	12	$\frac{2,330}{1,016}$	0.421	1.263	40.2° 39.6°	<i>Drugs:</i> codein 64 mg. <i>Food:</i> water 2,500 c.c., milk 150 c.c., broth 500 c.c., albumen 50 c.c., orange juice 350 c.c., soup 200 c.c., broth 500 c.c., cocoa 350 c.c.
Dec. 11	13	$\frac{2,200}{1,016}$	0.387	1.161	40.8° 39.5°	<i>Drugs:</i> calomel 64 mg., magnesium sulphate 15 gm., codein 32 mg., morphia 22 mg. <i>Food:</i> water 3,000 c.c., milk 350 c.c., lemon juice 180 c.c., broth 900 c.c., albumen 30 c.c.
Dec. 12	14	$\frac{1,750}{1,016}$	0.321	0.963	40.0° 39.8°	<i>Drugs:</i> calomel 196 mg., morphia 22 mg. <i>Food:</i> water 3,000 c.c., milk 200 c.c., lemon juice 30 c.c., soup 200 c.c., albumen 30 c.c., broth 900 c.c.
Dec. 13	15	$\frac{2,030}{1,017}$	0.374	1.122	40.5° 39.4°	<i>Drugs:</i> morphia 11 mg. <i>Food:</i> water 1,700 c.c., milk 800 c.c., broth 300 c.c., soup 150 c.c., orange juice 300 c.c., lemon juice 20 c.c.
Dec. 14	16	$\frac{1,770}{1,016}$	0.317	0.951	40.2° 39.5°	<i>Drugs:</i> morphia 11 mg., codein 32 mg. <i>Food:</i> water 2,000 c.c., milk 550 c.c., broth 650 c.c., orange juice 100 c.c., lemon juice 15 c.c.
Dec. 15	17	$\frac{1,510}{1,016}$	0.287	0.861	40.3° 39.6°	<i>Drugs:</i> calomel 96 mg., codein 48 mg. <i>Food:</i> water 2,000 c.c., milk 200 c.c., broth 670 c.c., orange juice 190 c.c., albumen 30 c.c.
Dec. 16	18	$\frac{1,600}{1,016}$	0.309	0.927	40.3° 39.6°	<i>Drugs:</i> codein 24 mg. <i>Food:</i> water 2,000 c.c., milk 400 c.c., broth 500 c.c., albumen 30 c.c., orange juice 280 c.c., ice cream 60 gm., lemon juice 10 c.c., soup 150 c.c.
Dec. 17	19	$\frac{1,610}{1,018}$	0.352	1.066	40.0° 39.0°	<i>Drugs:</i> none. <i>Food:</i> water 1,700 c.c., milk 600 c.c., broth 550 c.c., albumen 30 c.c., orange juice 200 c.c. Patient was operated on a few days later.

TABLE IX.

K. K., White Male Patient, Age Twenty-Three Years, Weight Fifty-Nine Kilograms.

Diagnosis: Bronchopneumonia.

Date.	Day of disease.	Urine in c.c. Specific gravity at 15°C.	Furfurophloroglucid in gm.	Glucuron in gm.	Rectal temperature, C. Maximum. Minimum.	Intake and remarks.
1913 Jan. 10	12	<u>900</u> 1,025	0.254	0.762	39.0° 39.0°	<i>Drugs:</i> codein 24 mg. <i>Food:</i> water 1,300 c.c., milk 800 c.c., orange juice 180 c.c., albumen 30 c.c., cocoa 100 c.c., egg 1.
Jan. 11	13	<u>900</u> 1,029	0.243	0.729	39.5° 38.9°	<i>White blood cells:</i> 8,000. <i>Drugs:</i> codein 24 mg. <i>Food:</i> water 600 c.c., milk 1,600 c.c., orange juice 300 c.c., cocoa 150 c.c., soup 150 c.c., egg albumen 30 c.c.
Jan. 12	14	<u>520</u> 1,030	0.149	0.447	39.2° 38.0°	<i>Drugs:</i> codein 24 mg. <i>Food:</i> water 1,600 c.c., milk 500 c.c., orange juice 300 c.c., soup 200 c.c., cocoa 300 c.c., albumen 30 c.c.
Jan. 13	15	<u>1,430</u> 1,028	0.350	1.050	38.0° 37.0°	<i>Drugs:</i> codein 16 mg. <i>Food:</i> water 1,600 c.c., milk 800 c.c., cocoa 480 c.c., orange juice 270 c.c., albumen 20 c.c.
Jan. 22	24	<u>1,120</u> 1,023	0.204	0.612	37.0°	<i>Food:</i> light diet,—milk, eggs, bread, and toast.
Jan. 23	25	<u>1,110</u> 1,020	0.188	0.564	37.5° 37.0°	<i>Food:</i> light diet,—milk, eggs, bread, and toast.
Jan. 24	26	<u>900</u> —	0.168	0.504	37.0° 36.8°	<i>Drugs:</i> codein 96 mg. <i>Food:</i> light diet,—milk, eggs, bread, and toast.
						Patient recovered. He was discharged on Feb. 17.

TABLE X.

O. L., White Male Patient, Age Twenty-Six Years, Weight Sixty-Three Kilograms.

Diagnosis: Influenza; Bronchitis, Acute.

Date	Day of disease.	Urine in c.c. Specific gravity at 15° C.	Furfural- phloro- glucid in gm.	Glucuron in gm.	Rectal temperature, C. Maximum. Minimum.	Intake and remarks.
1912 Dec. 3	4	$\frac{950}{1,016}$	0.176	0.528	39.9° 38.0°	20 hr. specimen. <i>Drugs:</i> codein 32 mg. <i>Food:</i> water 1,400 c.c., milk 1,100 c.c., eggs 4, soup 150 c.c., orange juice 15 c.c., toast 5 pieces, rice 30 gm., cereal 40 gm., custard 30 gm.
Dec. 4	5	$\frac{870}{1,017}$	0.142	0.436	38.4° 37.3°	<i>Drugs:</i> calomel 64 mg., magnesium sulphate 15 gm., codein 32 mg. <i>Food:</i> water 1,300 c.c., milk 1,200 c.c., orange juice 15 c.c., eggs 2, cereal 60 gm., potatoes 30 gm., custard 60 gm., pears 2, pear juice 40 c.c., toast 3 pieces.
Dec. 5	6	$\frac{950}{1,020}$	0.151	0.453	37.8° 37.0°	<i>Drugs:</i> none. <i>Food:</i> water 1,500 c.c., milk 1,300 c.c., orange juice 100 c.c., rice 30 gm., bread and toast 10 pieces, cereal 60 gm., eggs 2, bread pudding 75 gm., parsnips 30 gm.
Dec. 6	7	$\frac{1,430}{1,020}$	0.157	0.571	37.0° 36.8°	<i>Drugs:</i> none. <i>Food:</i> water 900 c.c., milk 1,200 c.c., orange 1, crackers 2, tapioca pudding 30 gm., potatoes 30 gm., rice 30 gm., squash 30 gm., custard 45 gm., eggs 4, cereal 60 gm., bread and toast 7 pieces. Patient recovered. He was discharged Dec. 11th.

TABLE XI.

Rabbit I. Previously Fed on Cabbage and Bread. Weight at the Beginning of the Experiment, Two Thousand Grams.

Date.	Weight in gm.	Urine in c.c. Specific gravity at 15° C.	Furfurophloroglucid in gm.	Glucuron in gm.	Rectal temperature, C.	Remarks.
1913 Jan. 16	1,820	<u>215</u> 1,014	0.0495	0.1485	39.8°	Intake: water 100 c.c.
Jan. 17	1,750	<u>135</u> 1,013	0.0223	0.0669	39.2°	Intake: water 100 c.c.
Jan. 18	1,600 (dead)	<u>130</u> 1,012	0.0318	0.0954	40.2°	10 hour specimen. Intake: water 50 c.c.; 0.5 c.c. of pneumococcus broth culture intravenously. Death followed after 10 hrs. Blood contained numerous pneumococci.

TABLE XII.

Rabbit II. Previously Fed on Cabbage and Bread. Weight at the Beginning of the Experiment, Two Thousand Five Hundred Grams.

Date.	Weight in gm.	Urine in c.c. Specific gravity at 15° C.	Furfurophloroglucid in gm.	Glucuron in gm.	Rectal temperature, C.	Remarks.
1913 Jan. 16	2,250	<u>260</u> 1,013	0.0447	0.1341	39.0°	Intake: water 100 c.c.
Jan. 17	2,120	<u>165</u> 1,008	0.0190	0.0570	39.0°	Intake: water 100 c.c.
Jan. 18	2,070	<u>74</u> —	0.0494	0.1482	40.2°	Intake: water 50 c.c.; 0.5 c.c. of pneumococcus broth culture intravenously.
Jan. 19	1,900	<u>140</u> 1,021	0.0218	0.0664	41.0°	Intake: water 50 c.c.; died 12 hrs. after collection of this specimen. Blood contained numerous pneumococci.

DISCUSSION.

On analyzing the cases of lobar pneumonia here reported, one sees that the amount of glycuronic acid excreted in twenty-four hours during the febrile period is, with but few exceptions (tables IV, VI, and VII), more than 0.8 of a gram. Often it is in excess of one gram, reaching sometimes a value of 1.3 grams. Bearing in mind the fact that a normal person of the same weight as the patients studied usually excretes daily on a moderate mixed diet 0.4 to 0.6

of a gram of glycuronic acid, one is justified in drawing the conclusion that there is an increase in the twenty-four hour output of this acid during the febrile period of pneumonia. The amount of increase varies between 0.2 and 0.9 of a gram. In only two cases out of twelve (tables VI and VII) was it impossible to determine a definite increase during the fever.

The question arises as to whether the increased output is perhaps attributable to some special ingredients of the diet, or to the drugs that have been administered to the patient during this period. Inasmuch as the figures show that a direct proportion between the output of fluid and the amount of glycuronic acid excreted is found only within wide limits, such a mechanical factor cannot be held responsible for the increased values observed.

The diet during the period of increased excretion of glycuronic acid consisted mainly of water, milk, orange juice, and albumen water. Some doubt arose as to whether the orange juice might cause an increased output in the urine of substances yielding furfurol. It may be seen in the protocols, however, that the increased excretion of glycuronic acid and the intake of orange juice do not vary together. Several additional experiments were also made on patients convalescing from lobar pneumonia after the resolution of the exudate, in order to see if the orange juice has a definite effect on the amount of glycuronic acid excreted. They were given 200 to 500 cubic centimeters of orange juice daily. The figures obtained, however, did not vary appreciably during the period of observation (table XIII).

TABLE XIII.

K. G., Colored Boy, Eight Years Old, Weight Thirty Kilograms, Convalescing from Lobar Pneumonia.

Date.	Urine in c.c. Specific gravity at 15° C.	Furfurol- phloroglucid in gm.	Glucuron in gm.	Intake.
1913 April 3	490 1.029	0.135	0.405	Milk, bread, potatoes, and moderate amount of meat.
April 4	510 1.031	0.154	0.462	Same diet plus 200 c.c. orange juice.
April 5	520 1.030	0.134	0.402	Same diet without orange juice.
April 6	440 1.031	0.129	0.387	Same diet plus 300 c.c. orange juice.

Some of the patients were given lactose (tables III and IV). In these cases, also, there is no evidence that lactose causes an increase in the urine of bodies that give rise to furfural. The excretion of glycuronic acid was not higher than in patients not receiving lactose. It may be mentioned, however, that in one fatal case the patient's urine contained a large amount of substances reducing Fehling's solution. Phloroglucin gave, with the distillate, a reddish brown instead of a black precipitate. It was very abundant,—several grams in the twenty-four hour specimen. The results have been discarded.

The patients on whom the observations were made received as drugs only small amounts of codein (not more than 96 milligrams) and morphia (not over 24 milligrams a day). Occasionally they were given calomel and magnesium sulphate. The cases in which larger amounts of glycuronic acid-combining substances were administered (camphor oil, salicylic acid, turpentine) have not been used. The excretion of glycuronic acid in those cases was still higher (table III). It has been also repeatedly found that the intake of 96 milligrams of codein in twenty-four hours does not provoke a marked increase in the output of substances giving rise to furfural (table IX). Furthermore, in the case of most of the patients studied, the excretion of glycuronic acid has also been estimated during the period of convalescence. The figures obtained in all the cases were lower than those observed during the active pathological process. They are sometimes high (tables I, II, and III), but this is undoubtedly due to the rich diet, especially to the intake of large quantities of meat. The more the diet during convalescence approaches that of the febrile period, the more evident the differences become (tables III, IV, and IX).

From the analytical data it seems justifiable to conclude that the increase in the excretion of glycuronic acid during the febrile period of lobar pneumonia is not due to outside factors, but that it is associated with the pathological process in the body. No accurate observations have been made concerning the length of time during which there is an increased output of glycuronic acid. Probably in most cases the figures return gradually to normal within a few days after the crisis or lysis.

This phenomenon is not peculiar to lobar pneumonia. Similar

observations have been made in postpneumonic empyema (table VIII) and in bronchopneumonia (table IX). However, in a case of influenzal bronchitis (table X), one day after the subsidence of the fever, the amount of glycuronic acid excreted was normal.

The variation in the output of glycuronic acid in general pneumococcus infections in rabbits was studied (tables XI and XII). The experiments were carried out on fasting animals. As seen in the tables, there is also in these cases a marked increase in the output of substances giving rise to furfural during the active period of the disease.

SUMMARY AND CONCLUSIONS.

1. It has been found that the amount of glycuronic acid excreted in the urine, estimated by Tollens's furfural distillation method, is increased during the febrile period of almost all cases of lobar pneumonia in man. Patients weighing from fifty-five to seventy kilograms excrete during the febrile period 0.8 to 1.3 grams of glycuronic acid in twenty-four hours, while the output of a normal individual of the same weight, age, and under similar conditions would not exceed 0.4 to 0.6 of a gram.

2. This increased output is not dependent on outside factors; it is attributable to pathological changes of the organism itself during the disease.

3. Similar observations have been made in postpneumonic empyema and bronchopneumonia.

4. Fasting rabbits, with general pneumococcus infection, have also shown a marked increase in the output of glycuronic acid during the pathological process.

Whether the conjugated substances with the glycuronic acid in the cases studied have the same origin and are qualitatively the same as under normal conditions, or whether we are dealing with different compounds, has not yet been determined.

A BIOLOGIC CLASSIFICATION OF PNEUMOCOCCI BY MEANS OF IMMUNITY REACTIONS.*

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The early work of Koch on etiology led him to formulate certain laws which he demanded should be fulfilled before the relationship of any micro-organism to disease could be regarded as satisfactorily established. Subsequent students have sought to fulfil the requirements of Koch, and the result has been that with the elucidation of each well-recognized infection, a single bacteriologic unit has become accepted as the causative agent. Bacteria, however, are not strictly limited in the pathologic process which they induce, and the lesion caused by a single variety may vary somewhat with its location in the animal body. The pathologic and clinical study of disease is, however, so extensive to-day that the various types of activity of the same bacterial race have been thoroughly described and correlated, so that the nature of the infectious agent can in most instances be assumed from the character of the pathologic process.

From the demonstration by Pasteur of the causal relationship of bacteria to disease, and the evidence of Koch that most infections are caused by bacteriologic units, has come the hope that at some time bacterial diseases may be controlled by specific methods. The increasing study of bacteriology and the practical application of bacteriologic immunity to human disease have made it evident that, in addition to the grosser classification of which bacteria are susceptible, certain finer characteristics exist which serve to differentiate still further members of the same bacterial species. In some instances, certain growth phenomena, such as cultural or fermentative reactions, are sufficient for differentiation; others require the

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extraordinarily specific methods that have developed from the study of immunity. Although, from the point of view of etiology, the drawing of such fine lines of division may be relatively of minor significance, from that of specific therapy these differences are of primary importance. How numerous and important are the varieties of the main bacterial races is not very well known at present. Most investigators recognize the probability of such a condition, and in the specific bacterial and serum therapy of the day, an attempt is made to cover all possibilities by the use of polyvalent vaccines and immune serums. Such serums and vaccines are, however, prepared without much forethought; heterogeneous collections of organisms are used, and difference in origin is the only standard of differentiation of the various strains employed. In such a method, many individuals may be superfluous, and, on the other hand, some important members of the species may be lacking. In this paper we shall report the results of a biologic classification of the pneumococcus, using in the main certain immune reactions for purposes of differentiation.

For the past three years, we have been engaged in a study of lobar pneumonia at the Hospital of the Rockefeller Institute. The problem in the beginning was approached with a view of obtaining, if possible, a specific therapy. The work of Neufeld¹ and his associates has demonstrated the existence of certain varieties of pneumococcus which, in regard to certain of their immunologic reactions, are fundamentally different. In recognition of the importance of this fact, a study of all pneumococci obtained from human cases has been made. Immune reactions have been chosen for purposes of differentiation because of their peculiar specificity and important practical bearing, and because of the inconstancy of morphologic and cultural differences of strains of pneumococcus. A primary classification has been obtained by means of protection experiments. All the strains of pneumococcus, as soon after isolation as sufficient virulence was attained, have been tested for their susceptibility to the action of serums prepared by immunization of animals to single varieties. White mice were used as experimental animals, and the various mixtures of serum and culture were injected simultaneously

1. Neufeld and Haendel: *Arb. aus d. kais. Gesund.*, 1910, **xxxiv**, 293.

into the peritoneal cavity. The results were seldom doubtful. If the organism tested belonged to the same group as that from which the serum had been derived, as great a degree of protection was usually afforded as that manifested against the homologous organism. On the other hand, when a strain belonged to a different group, little or no protection was evident, the tested animals dying for the most part within the same time as the controls. The results obtained from testing different strains in the manner described are shown in Table I.

TABLE I.
Classification by Protection.

	No	Per Cent.
Group 1.....	28	45
Group 2.....	12	20
Group 3 (mucosus).....	9	14
Group 4 (heterogeneous).....	13	22
Total typical.....	49	78
Total heterogeneous.....	13	22
Total.....	62	

In this table are enumerated in detail the results obtained from testing the protective power of two different immune serums derived from typical organisms. Preliminary work had previously shown that the two strains of pneumococcus chosen for immunization were probably the ones most frequently associated with lobar pneumonia. In the main the differentiation obtained by protection is sufficiently evident to permit a satisfactory classification. In all, sixty-two different strains have been tested against the two types of immune serums. Of the sixty-two strains, forty, or 64.5 per cent., were susceptible to the action of one or the other type of immune serums. On the other hand, twenty-two strains were either completely unaffected by the immune serum or so little influenced as to render inclusion in either of the typical groups unjustifiable. Of the organisms which could be classified by the protection reaction, twenty-eight, or 45 per cent., of the whole number of strains studied responded to the protection of serum Type 1, and shall be called Group 1. The Type 2 serum exhibited protection against twelve

strains, 20 per cent. of the whole number studied. These organisms are classed as Group 2. Of the twenty-two strains not materially influenced by the protective action of the serum, nine were easily differentiated by their distinguishing cultural and pathogenic qualities into a group which we have called Group 3, exhibiting the characters of the so-called *Streptococcus* or *Pneumococcus mucosus*. These organisms are highly pathogenic for white mice and are completely insusceptible to the action of either of the immune serums in our possession. The remaining thirteen strains must be considered as forming a heterogeneous group, designated as Group 4. These organisms do not possess cultural characters that evidently distinguish them from either of the typical groups, and, although one or another of the serums studied may show slightly increased protection, this is so small when compared with the known protective value of the serum that it does not justify inclusion in the typical groups. These differences are further emphasized later in the discussion of the results of agglutination experiments. The results of the work on protection show the existence of four general groups of organisms as the causative agents of lobar pneumonia. Groups 1 and 2 are made up of organisms that are identical from the point of view of protection with other members of their respective groups. Group 3, *Pneumococcus mucosus*, is formed of organisms having distinctive growth characters. Whether or not further classification of this group is possible by means of immunity reactions has not yet been determined because of the difficulty of obtaining satisfactory immune serums against these organisms. Group 4 has no completely distinctive morphologic or cultural characters, and, as is shown later, consists of a heterogeneous collection of isolated individuals.

AGGLUTINATION.

A historical discussion of the studies of agglutination of pneumococcus does not seem necessary in this paper. It is sufficient to state that the pneumococcus is agglutinable in specific immune serums, and that evidence in regard to the demonstration of the existence of different varieties by this reaction is contradictory. Pneumococcus does not always agglutinate readily in high dilu-

tions of immune serums. Fortunately, however, it has the property of remaining suspended or growing diffusely in practically pure normal or heterologous serums, so that serum of considerable strength can be used to bring out specific differences. In the following study, serum and twenty-four-hour broth-cultures of the organism were mixed together in equal quantities. The reactions were read by the macroscopic method after one or two hours at 37 C. (98.6 F.), and again after twenty-four hours at either 37 C. or room temperature. Agglutination usually begins to be visible in from fifteen minutes to half an hour, and appears first as a fine granulation of the suspension; the clumps finally sink to the bottom of the tube and in the end usually form a firm, scale-like mass in the bottom of the tube which cannot be broken up, even by vigorous shaking. If the mixtures are allowed to stand for twenty-four hours at 37 C., a precipitation of serum and organisms sometimes occurs in the control tubes which, however, after a little experience is easily distinguishable from a true agglutination. In control tubes, even when such a precipitation occurs, the majority of the cocci remain freely suspended, whereas when specific agglutination develops, the supernatant fluid always becomes perfectly clear. The organisms studied were tested against the same two types of serums used for the protection experiments, and the results obtained are shown in the Table 2.

TABLE 2.
Classification by Agglutination.

	No.	Per Cent.
Group 1.....	22	41
Group 2.....	11	20
Group 3 (mucosus).....	9	17
Group 4 (heterogeneous).....	12	22
Total typical.....	42	77
Total heterogeneous.....	12	23
Total.....	54	

Study of the agglutination reactions of these strains of pneumococci shows that specific differences can be brought out by this method between individual members of a collection of organisms.

The strains again fall into definite related groups, and the percentage of distribution is about the same as that obtained in protection experiments. In every instance in which an organism could be definitely placed by protection in one of the groups described, the agglutination reaction has corresponded with this classification. Two strains, 56 and 86, showed doubtful protection with both types of serums, but on agglutination reacted specifically with serum Type 1.

By the methods of protection and agglutination, it has been possible to classify the pneumococci associated with lobar pneumonia in man into four groups. Groups 1 and 2 consist of members closely related immunologically to other members of their respective groups. Group 3 is formed of *Pneumococcus* or *Streptococcus mucosus*. Of nine members of this group studied, eight possessed the common characters of pneumococcus in addition to the quality of forming mucus, and one those of streptococcus. The latter organism was insoluble in bile, failed to ferment inulin, hemolyzed blood mediums, and exhibited other characteristics of *Streptococcus longus*. It formed, however, abundant mucous exudate in animals, grew in large mucoid colonies and showed well-developed capsules. A study is now in progress to determine whether or not immunologic differences exist between individual members of the mucous group. The fourth group consists of heterogeneous members, and a further study of this group appears later. All the strains of pneumococcus examined were studied by the usual cultural methods. Although average cultural and morphologic differences exist between the different groups, these are variable and are not reliable for purposes of identification, except those of *Pneumococcus mucosus*. All the strains studied, except the *Streptococcus mucosus* mentioned before, were either bile-soluble on isolation or have become so on animal passage. Fermentation of inulin is not more characteristic of one group than another. Capsules are somewhat easier to demonstrate among members of Group 1 and *mucosus* than in Group 2 and the heterogeneous group. The average morphology of the various groups shows some differences. In Group 1, individual cocci are medium-sized, slender and show a slight tendency to rounded ends. Individuals of Group 2 are larger, fairly broad and almost invari-

ably show perfect lancet-shaped forms. The morphology of *Pneumococcus mucosus* is quite characteristic, showing voluminous capsules containing medium-sized, closely approximated cocci with definitely rounded ends. In general, members of the heterogeneous group tend to exhibit pairs of rounded individuals, but may resemble completely either Group 1 or Group 2. The virulence for mice of strains of the typical Groups 1 to 3 is usually higher and more constant than that of the heterogeneous Group 4. A continuous study of a number of members of the different groups may thus bring out certain more or less characteristic differences. These, however, are not constant, and cannot be depended on for purposes of classification.

The frequency with which the different types of pneumococcus appear as the cause of lobar pneumonia is of considerable interest. Altogether it has been possible to obtain a biologic classification of the pneumococci from seventy-four cases of typical lobar pneumonia. In thirty-five instances, 47 per cent., the organisms belonged to Group 1; in thirteen instances, 18 per cent., to Group 2; in ten instances, 13 per cent., to Group 3 (*mucosus*) and in sixteen instances, 22 per cent., to Group 4 (heterogeneous). Almost one-half the cases of pneumonia which we have seen are due to pneumococci belonging to Group 1. Members of the other groups occur less frequently. In 78 per cent. of the cases examined, the organisms obtained have possessed distinguishing characteristics which permit a definite biologic grouping. The remainder do not have distinctive common immunologic or growth characters and must be classed as heterogeneous. Immune serums have been prepared with organisms from Groups 1 and 2, and apparently all members of these two groups are susceptible to the protective and agglutinative action of these serums. The immunity reactions of Group 3 (*mucosus*) have not as yet been completed.

Group 4 has been called heterogeneous. The reason for this classification is shown in the following study: Animals were immunized to various members of this group and the immune reactions which had been found to be common to members of Groups 1 and 2 were studied. So far, it has not been possible to protect animals against any of the heterogeneous strains, when serum de-

rived from another strain has been used. The immune serums obtained protected against only the homologous organism. An example of the method employed is shown in Table 3. The immune serum was obtained by immunization of an animal to *Pneumococcus* 71. The cultures used for infection were twenty-four-hour broth-cultures, freshly obtained from passage through animals. Culture and serum were injected simultaneously into the peritoneal cavity of white mice.

Certain other strains belonging to the heterogeneous group have been tested in the same manner, and in no instance has any cross-protection been observed. From Table 3 it is seen that not only does Serum 71 fail to afford any protection against heterologous strains, but in some instances even increases the fatality of the smaller doses of culture. The serum, however, possessed a high protective power against the homologous organism. The condition of affairs is, therefore, quite different in this group from that observed in Groups 1 and 2. In the latter a serum derived from one member of the group seems to protect animals against infection with all other members of the group, whereas in the former, no instance of cross-protection has been observed.

In addition to the protection reactions, cross-agglutination among members of this group has been carefully studied. The technic employed was the same as that previously described. The results are shown in Table 4.

In this table the results obtained from testing fourteen different strains against serums derived from eleven of the strains are presented. The agglutination reaction has again confirmed the results of protection experiments. In only one instance was cross-agglutination observed, *Pneumococcus* 55 by the polyvalent serum obtained from *Pneumococci* 34, 38 and 62. In two other instances, *Pneumococci* 37 and 52, agglutination occurred in heterologous serum, but the reaction took place much more slowly than that of the homologous organisms. The study of cross-protection and agglutination among members of the heterogeneous Group 4 indicates that this group comprises a number of distinct varieties of pneumococcus which cannot be related to one another by immuno-

logic reactions. Culturally they are true pneumococci and manifest all the common characters of pneumococcus.

The study of certain biologic reactions of a large number of strains of pneumococci isolated from cases of pneumonia in man has developed some interesting facts. The organisms studied fall into two general groups. One of these, containing a large majority of all strains investigated, consists of three apparently fixed races, and the other of a smaller number of strains, each of which seems to form a distinct variety. The individual members of two of the fixed races (Groups 1 and 2) can be distinguished certainly from one another only by means of highly specific immune reactions. Single strains, however, belonging to either of these two groups have immune reactions in common with all other members of the particular group to which they belong. The other apparently fixed race consists of the organisms designated as *Pneumococcus mucosus*. Although the latter classification has been based on cultural reactions and does not exclude the existence of different varieties, from the point of view of immunity, incomplete immunologic studies indicate that most of these organisms can also be further related by means of immunity reactions. In striking contrast to these three groups is the fourth or heterogeneous group, which seems to consist entirely of isolated individuals. The significance of such a situation is difficult to interpret. Whether this mixture is being progressively differentiated into groups with common characters, or whether each member is a descendant of one of the fixed groups that has undergone a fundamental change by being forced to adapt itself to some special environment, cannot be determined from the evidence at hand. It is not impossible that the fixed races are more highly parasitic and are never very far removed from a condition of pure parasitism, whereas the heterogeneous strains may be representatives of the type of pneumococcus found in the normal mouth and consequently more likely to have undergone environmental changes.

The specificity of the immunity reactions has been very striking and, from the practical point of view of specific therapy, is of great importance. A large amount of work has been devoted to

study of the agglutination reactions of pneumococcus. Kindborg² has found that satisfactory agglutination occurs practically only when homologous serums are used. On the other hand, Hiss³ has obtained a wide degree of cross-agglutination. Such contradictory results may largely depend on the investigator's luck in securing suitable organisms. If the collection should consist of a majority of members of one of the fixed groups, cross-agglutination would be the rule, whereas if the heterogeneous strains should predominate, a high degree of individual specificity would be observed. The fact that cross-protection does not occur except among members of individual groups of the fixed races has an important practical bearing. If polyvalent serums are to be used in the therapy of lobar pneumonia, such serums must be derived from representative members of the typical groups, if the therapeutic application is to be successful. On the other hand, if one uses univalent serums, representing each of the fixed races, a diagnosis of the type of organism concerned in individual instances of the disease must be made. The latter method is quite practicable and has been successfully employed at the Hospital of the Rockefeller Institute during the past year. Whether or not the results obtained from this study of pneumococci can be profitably applied to other pathogenic bacteria cannot be definitely asserted. It is not, however, illogical to assume that such a classification of other infectious organisms could be made. If this is true, in order that specific bacterial and serum therapy may be intelligently applied, such a study becomes imperative.

2. Kindborg: Diss. (Halle), Leipsic, 1905. Ref. *Centralbl. f. Bakt.*, 1906, Part I, xxxviii, 172.

3. Hiss: *Jour. Exper. Med.*, 1905, vii, 547.

THE EFFECT OF INTRASPINOUS INJECTIONS OF SALVARSAN AND NEOSALVARSAN IN MONKEYS.*

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The use of intraspinous injections of antimeningococcus serum in the treatment of epidemic cerebrospinal meningitis (1), and the increased efficiency of serum so used over serum injected intravenously, has established the value of local therapy in the treatment of this infectious disease of the central nervous system. The desirability of obtaining a method of local application of curative agents in syphilitic infections of the nervous system therefore at once suggests itself.

Horsley (2) in 1910 advocated washing out the cerebrospinal canal, in cases of syphilitic disease, with 1 to 1,000 solutions of bichloride of mercury. This method, however, was soon abandoned. The well known irritating properties of salvarsan when injected into the tissues prohibited the use of this drug in strong concentrations. It was considered desirable, however, to investigate the possibility of the use of minute quantities of salvarsan and neosalvarsan intraspiously, and the intraspinous injection of small quantities of these drugs was therefore performed in monkeys. The results obtained are presented in this communication.

TECHNIQUE.

Macacus rhesus and *Macacus nemestrinus* monkeys were used in the experiments. Under ether anesthesia lumbar puncture was performed and a small quantity of spinal fluid withdrawn. By means of a two cubic centimeter Luer glass syringe, the substance to be tested was then slowly injected through the lumbar puncture needle.

* Received for publication, June 27, 1913.

TABLE I.

No. of animal.	Day of experiment.						Remarks.
	1st.	3d.	4th to 8th.	10th.	12th.	13th to 18th.	
<i>Macacus rhesus</i> 1	Cell count 4. Intra-spinous injection of 8 mg. salvarsan in 2 c.c. 40% human serum	Cell count 1,100				Cell count 450 (16th day)	Recovered. Died six weeks later of pulmonary tuberculosis. The lumbar and dorsal portions of the cord showed some superficial congestion.
<i>Macacus rhesus</i> 2	Bloody fluid. Intra-spinous injection of 3.2 mg. salvarsan in 2 c.c. 40% human serum	Dry tap	Paralysis of legs began on 3d day and became complete	Dry tap			No recovery from paralysis. Killed.
<i>Macacus rhesus</i> 3	Cell count 55. Intra-spinous injection of 1.6 mg. salvarsan in 2 c.c. 40% human serum	Cell count 1,180 (2d day)	Cell count 1,600 (5th day)			Cell count 260 (13th day)	Reinjected two months later and died during injection. Death suggested human serum anaphylactic shock. Brain and cord normal.
<i>Macacus rhesus</i> 4	Cell count 6. Intra-spinous injection of 1 mg. salvarsan in 1.6 c.c. 40% monkey serum	Cell count 1,280	Cell count 2,000 (8th day)	Died, miliary tuberculosis			Marked subpial congestion of lumbar and dorsal cord; no tubercles seen in brain or cord.

TABLE I—Continued.

No. of animal.	Day of experiment.							Remarks.
	1st.	3d.	4th to 8th.	10th.	12th.	13th to 18th.	20th.	27th.
<i>Macacus rhesus</i> 5	Cell count 3. Intraspino- us injection of 0.4 mg. salvar- san in 1.6 c.c. 40% monkey serum	Cell count 890	Cell count 8 (8th day)			Cell count 6. Intraspino- us injection of 0.4 mg. sal- varsan in 1.6 c.c. 40% monkey serum (18th day)	Cell count 2,510	Cell count 69 Recovered.
<i>Macacus ne- mestrinus</i> 6	Cell count 90. In- traspino- us in- jec- tion of 0.2 mg. salvarsan in 1.6 c.c. 40% monkey serum	Cell count 855		Cell count 544				Recovered. Died six months later of tuber- culosis. Brain and cord normal.
<i>Macacus ne- mestrinus</i> 7	Cell count 29. In- traspino- us in- jec- tion of 0.1 mg. salvarsan in 1.6 c.c. 40% monkey serum	Cell count 405		Cell count 104		Died (13th day)		Pulmonary tuberculosis, chronic nephritis. Brain and cord nor- mal.
<i>Macacus rhesus</i> 8	Cell count 10. In- traspino- us in- jec- tion of 0.3 mg. neosalvarsan in 1.6 c.c. 40% mon- key serum	Cell count 334		Cell count 66. In- traspino- us in- jec- tion of 1.5 mg. neosalvarsan in 1.6 c.c. 40% mon- key serum	Cell count 4,082			Recovered.

No. of animal.	Day of experiment.								Remarks.
	1st.	3d.	4th to 8th.	10th.	12th.	13th to 18th.	20th.	27th.	
<i>Macacus rhesus</i> 9	Cell count 35. Intraspineous injection of 0.15 mg. neosalvarsan in 1.6 c.c. 40% monkey serum	Cell count 50		Cell count 36. Died under ether					Brain and cord normal.
<i>Macacus rhesus</i> 10	Cell count 98. Intraspineous injection of 0.045 mg. neosalvarsan in 1.6 c.c. 40% monkey serum	Cell count 335		Cell count 60. Intraspineous injection of 0.15 mg. neosalvarsan in 1.6 c.c. 40% monkey serum	Cell count 84				Recovered.
<i>Macacus rhesus</i> 11	Cell count 122. Intraspineous injection of 0.45 mg. neosalvarsan in 1.6 c.c. 0.9% sodium chloride	Cell count 184							Recovered.
<i>Macacus rhesus</i> 12	Cell count 14. Intraspineous injection of 0.9 mg. neosalvarsan in 1.6 c.c. 0.9% sodium chloride	Cell count 351							Recovered. Died two months later of miliary tuberculosis. Brain and cord normal.

The salvarsan solutions used consisted of one cubic centimeter of the alkaline solution of the drug in 0.9 per cent. sodium chloride, and 0.6 of a cubic centimeter of human or normal monkey serum, giving a dilution of serum to 40 per cent. Solutions of neosalvarsan in 0.9 per cent. sodium chloride were similarly injected in 40 per cent. monkey serum. In two monkeys injected with neosalvarsan, the serum was omitted and a simple solution of the drug in 0.9 per cent. sodium chloride was used. In three monkeys second injections were also made.

In judging the irritating properties of these various substances, the cell count in the spinal fluid was used as indicator. A count of the cells was made before treatment, two days after treatment, and again about a week later. The cell counts were made in the usual manner with a white blood-counting pipette and a Thoma-Zeiss chamber. The results of the experiments are given in table I.

The cell count in the spinal fluid of monkeys, before treatment had been begun, was found to vary within wide limits. In addition to those recorded in the table, four other normal monkeys were punctured to determine this point. Their spinal fluids showed cell counts of 10, 40, 85, and 137 per cubic millimeter. Apparently healthy monkeys, therefore, yielded fluids showing counts varying from 3 to 137 per cubic millimeter. This is in striking contrast to man, where the normal count varies from 0 to 5 per cubic millimeter, and in healthy individuals probably never exceeds the latter figure. The cell count following intraspinal injections also varied widely. The highest obtained was 4,082 per cubic millimeter (*Macacus rhesus* 8). This followed the injection of 1.5 milligrams of neosalvarsan, nine days after 0.3 of a milligram had been injected.

Reference to table I shows that the injection of even small quantities of salvarsan is very irritating. 3.2 milligrams caused in one monkey (*Macacus rhesus* 2) paralysis of the hind legs persisting for some months. The injection of one milligram of salvarsan and over produced in every case (*Macacus rhesus* 1, 3, and 4) cell counts of over 1,000 per cubic millimeter, and the injection of even 0.2 of a milligram produced quite a profound cellular reaction which still persisted ten days after the injection (*Macacus nemestrinus* 6).

0.1 of a milligram of salvarsan produced a much less severe reaction (*Macacus nemestrinus* 7).

The injections of neosalvarsan were, on the whole, less irritating, but *Macacus rhesus* 8 showed a severe reaction,—4,082 cells per cubic millimeter on the second injection.

One striking result was the apparent increase in the irritation produced by a second injection in another monkey (*Macacus rhesus* 5). 0.4 of a milligram of salvarsan produced on first injection a cell count of 890 per cubic millimeter; on reinjection, eighteen days later, the same amount of salvarsan was followed by a count of 2,570 cells per cubic millimeter. The increase in cells in these fluids following injections of salvarsan and neosalvarsan consisted almost entirely of polynuclear leucocytes.

Wechselmann (3) studied the effect of salvarsan when injected directly into the brains of dogs and rabbits. Injections of 0.5 to 1 c.c. of a concentration of 1:500 and 1:1,000, that is, of 1 mg. of salvarsan, caused convulsions, weakness of the limbs, and death in from two to four days. Concentrations, however, of 1:2,000 and 1:4,000, that is, 0.5 to 0.25 mg. of salvarsan, produced no disturbance.

In 1912 we reported on the injection of minute quantities of neosalvarsan intraspinaly in a case of *tabes dorsalis* (4). The severe lightning pains and temporary retention of urine which sometimes followed this procedure caused us to discontinue its use.

Wechselmann (5) has subsequently reported the results of intraspinal injections of neosalvarsan in four patients. One paretic was given 3 mg. of neosalvarsan intraspinaly and two weeks later 1 mg. There was no reaction. Another paretic was given 0.5 mg. Two children with congenital syphilis were also injected with from 1 to 1.5 mg. without any reaction occurring.

More recently Marinesco (6) has reported severe results from the use of neosalvarsan intraspinaly. Thirteen patients were injected, and in three the treatment was repeated. He used, as a rule, 4 c.c. of a 1:800 solution or about 5 mg. of neosalvarsan. While in one or two patients no reaction occurred, the reaction in the majority was severe, and in several cases more serious effects were observed. Thus, in eight patients there was incontinence of urine or else retention, requiring constant catheterization. In three cases there was weakness of the extremities, and in one anesthesia of the rectum, legs, and buttocks.

In a previous communication (7) we have described the use of intraspinal injections of salvarsanized serum in the treatment of syphilitic infections of the central nervous system. The method is to bleed patients one hour after an intravenous injection of salvarsan, separate the serum, and reinject this serum into the sub-

arachnoid space. In view of the favorable effects of the injections of this serum and the risk of causing injury by the direct injection of neosalvarsan, it seems advisable to confine our efforts at the local therapy of syphilis of the central nervous system with salvarsan to the use of such salvarsanized serum.

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A STUDY OF THE SPIROCHÆTICIDAL ACTION OF THE SERUM OF PATIENTS TREATED WITH SALVARSAN.*

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While studying, during the past two years,¹ the effects of the intraspinous mode of treatment of syphilis of the central nervous system, we have incidentally tested the effects of salvarsanized blood serum upon the spirochæta of relapsing fever.

Gonder² noted that the serum of rats injected intravenously with large doses of salvarsan exhibited a spirochæticidal effect on *Spirochata recurrentis*. The rat serum was permitted to act upon a suspension of spirochæta which were subsequently injected intraperitoneally into mice; the mice remained free of infection, while the controls injected with spirochæta acted on by normal rat serum developed infection. *Spirochata gallinarum* were similarly injured by the blood serum of hens intravenously injected with salvarsan, while intramuscular injections were less active. The spirochæticidal effect was observed only after the treated spirochæta were injected into animals, as no difference in motility between treated and untreated spirochæta could be demonstrated. The blood taken shortly after the salvarsan injection was most active, and the spirochæticidal effect disappeared in a few hours, but was parallel with the intensity of the P-dimethylamidobenzaldehyde reaction of the serum for salvarsan. Finally, the serum of salvarsan-treated rats exhibited a curative action in mice infected with *Trypanosoma brucei*.

METHODS.

In the present study *Spirochata duttoni*, propagated in white rats or mice, has been employed. On the third day after inoculation, the blood usually contained fifty or more spirochæta per field of the dark-field microscope. When the virulence of the spirochæta fell off, it was raised by more frequent animal passage. The suspension for performing a test was prepared by bleeding an infected mouse or rat into normal saline, and the blood was diluted

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¹ Swift, H. F., and Ellis, A. W. M., *New York Med. Jour.*, 1912, xcvi, 53.

² Gonder, R., *Ztschr. f. Immunitätsforsch., Orig.*, 1912, xv, 257.

with saline until the required number of spirochætæ per field was obtained. One cubic centimeter of the spirochæta suspension was mixed with one cubic centimeter of the serum or dilution of serum, as indicated in the protocols, the resulting mixture being well shaken and then incubated at 37° C. for one hour. One cubic centimeter of the mixture, representing 0.5 of a cubic centimeter of the spirochæta suspension, was injected into mice intraperitoneally. The blood of the inoculated mice was examined daily with the dark-field microscope and twenty fields of each preparation were counted. The average number of spirochætæ per field is indicated in the protocols. Control mice inoculated with the same amount of untreated spirochætæ or spirochætæ mixed with untreated serum were used. Only those experiments in which the controls showed a good infection were accepted.

PROTOCOL I.

The Action of the Serum of a Normal Rabbit Treated Intravenously with Neosalvarsan.

Mouse.	Rabbit serum obtained after treatment.	Dilution of serum.	Day after inoculation.				
			1	2	3	4	5
B-1	Immediately	1 : 1	0	0	0	0	0
B-2	Immediately	1 : 2	0	0	0	0	0
B-3	Immediately	1 : 4	0	±	+	0	0
C-1	1 hour	1 : 1	0	0	0	0	0
C-2	1 hour	1 : 2	0	± w	++	+++	0
C-3	1 hour	1 : 4	+	+++	+++	0	0
D-1	2 hours	1 : 1	± w	±	0	0	0
D-2	2 hours	1 : 2	+	+++	++	0	0
D-3	2 hours	1 : 4	++	+++	+	0	0
E-1	4 hours	1 : 1	± w	+	+	0	0
E-2	4 hours	1 : 2	+	++	0	0	0
E-3	4 hours	1 : 4	++	+++	+++	0	0
F-1	6½ hours	1 : 1	+	++	±	0	0
F-2	6½ hours	1 : 2	++	+++	+	0	0
F-3	6½ hours	1 : 4	++	++++	++++	0	0
Control							
A-1	Before	1 : 1	++	++++	++++	0	0

± w = between 1 and 19 spirochætæ in twenty fields.

± = 1 per field.

± = 2 to 10 per field.

++ = 11 to 20 per field.

+++ = 20 to 50 per field.

++++ = more than 50 per field; countless.

A normal rabbit was bled into centrifuge tubes before and at intervals after intravenous injections of a watery solution of neosalvarsan in the dose of 0.15 gm. (equivalent to 0.1 gm. salvarsan) per kilogram of body weight. The serum was allowed to separate over night and centrifugalized the next day. The spirochætal blood came from a rat inoculated three days previously; it was diluted to 20 c.c. with saline, so that each field contained between fifteen and eighteen spirochætæ. This suspension was mixed with various dilutions of the rabbit serum, incubated one hour and injected into mice.

The spirochætæ from each tube showed no diminution of motility under the dark-field microscope. The rabbit serum had a definite spirochæticidal effect, which was most marked in that taken immediately after the neosalvarsan injection and steadily diminished as the interval between treatment and bleeding increased, until it had nearly disappeared at the end of six and one half hours. The effect also disappeared in higher dilutions of the serum. Hence the action of the rabbit serum accords with the results obtained by Gonder.

PROTOCOL II.

The Action of the Heated Serum of a Rabbit Treated Intravenously with Neosalvarsan.

Mouse.	Rabbit serum obtained after treatment.	Dilution of serum.	Serum heated or unheated.	Day after inoculation.			
				1	2	3	4
B-1	Immediately	1 : 1	Unheated	0	0	0	0
B-2	Immediately	1 : 2	Unheated	0	0	0	0
B-3	Immediately	1 : 4	Unheated	0	+	0	0
B-4	Immediately	1 : 1	Heated	0	0	0	0
B-5	Immediately	1 : 2	Heated	0	0	0	0
B-6	Immediately	1 : 4	Heated	0	0	0	0
C-1	1 hour	1 : 1	Unheated	0	± w	0	± w
C-2	1 hour	1 : 2	Unheated	0	±	+	0
C-3	1 hour	1 : 4	Unheated	± w	+++	0	0
C-4	1 hour	1 : 1	Heated	0	0	0	0
C-5	1 hour	1 : 2	Heated	0	0	0	0
C-6	1 hour	1 : 4	Heated	0	0	0	0
D-1	2 hours	1 : 1	Unheated	0	+	± w	0
D-2	2 hours	1 : 2	Unheated	±	++	+	0
D-3	2 hours	1 : 4	Unheated	+	+++	+	0
D-4	2 hours	1 : 1	Heated	0	0	0	0
D-5	2 hours	1 : 2	Heated	0	0	0	0
D-6	2 hours	1 : 4	Heated	0	0	0	0
Controls							
A-1	Before	1 : 1	Unheated	++	++++	0	0
A-4	Before	1 : 1	Heated	++	++++	++	0
X-1	—	—	—	++	++++	++	0

A normal rabbit was injected intravenously with neosalvarsan in the dose of 0.15 gm. (= 0.1 gm. salvarsan) per kilogram of body weight; it was bled before, immediately after, and one and two hours after treatment. The separated serum was divided into two lots and one half of each lot was heated at 56° C. for thirty minutes. Various dilutions of the unheated and heated sera were incubated with a suspension of *S. duttoni* and injected into mice.

The human serum, injected intraspinaly into patients with syphilis of the central nervous system, is heated to 56° C. for one half hour, in order to kill any microorganisms which might be present. After observing that the serum of neosalvarsan-treated rabbits exerted a spirochæticidal action, we tested the effect of heating the serum upon its action on the spirochæta. Protocol II shows that instead of decreasing the spirochæticidal action, the heating markedly increased it. The mice inoculated with spirochætæ, treated with rabbit serum which was withdrawn one and two hours after the neosalvarsan injection, all developed some, but a less degree of, infection than the control animals; while the corresponding mice, injected with spirochætæ acted upon by heated serum, all remained free from infection. Moreover, the heated serum, taken two hours after the neosalvarsan injection, exerted as strong spirochæticidal action as did the unheated serum taken immediately after the injection, while the serum obtained from the rabbit before treatment (serum A) was devoid of action whether unheated or heated. Hence, the effect of heating was to increase the spirochæticidal action of the salvarsanized serum, and not to render more effective some spirochæticidal substance in normal serum. The serum of rabbits that received 0.075 of a gram of neosalvarsan per kilogram of body weight was diluted 1 to 5 and 1 to 10, as well as 1 to 2 and 1 to 4, and acted in the same manner.

PROTOCOL III.

Comparison of the Prophylactic and Curative Action of the Serum of a Neosalvarsan-Treated Rabbit.

Mouse.	Rabbit serum obtained after treatment.	Heated or unheated.	Serum injected.	Treatment at time of inoculation	Day after inoculation.				
					1	2	3	4	5
B-1	Immediately	Unheated	Intraperitoneally		0	0	0	0	0
B-2	Immediately	Heated	Intraperitoneally		0	0	0	0	0
C-1	1 hour	Unheated	Intraperitoneally		0	+	++	+++	+++
C-2	1 hour	Heated	Intraperitoneally		0	0	0	0	0

Mouse.	Rabbit serum obtained after treatment.	Heated or unheated.	Serum injected.		Day after inoculation.				
					1	2	3	4	5
Controls									
A—2	Before	Heated	Intraperitoneally		+	++	++	0	0
X	—	—	—		±	++	++	0	0
B—5	Immediately	Unheated	Intraperitoneally		±	++	++	0	0
B—6	Immediately	Heated	Intraperitoneally		±	+	0	0	0
C—5	1 hour	Unheated	Intraperitoneally		±	++	++	0	0
C—6	1 hour	Heated	Intraperitoneally		±	+	++	0	0

A normal rabbit was injected intravenously with neosalvarsan, 0.075 gm. (=0.05 gm. salvarsan) per kilogram of body weight, and bled before, immediately after, and one hour after treatment. The serum was divided and one half of each specimen was heated at 56° C. for thirty minutes. Mice of the same weight were each inoculated intraperitoneally with 0.5 c.c. of a suspension of infected mouse blood, so diluted that there were five to six spirochætæ per field. Part of the mice were immediately injected with 0.5 c.c. of various specimens of heated and unheated treated rabbit serum. The remainder were treated the following day, when all had developed a moderate infection; they received 0.5 c.c. of the same serum.

The prophylactic effect of the rabbit serum was more marked than the curative action. Since the spirochætæ and serum were not brought into contact in the test-tube, as in the previous experiments, the action of the serum was exerted *in vivo*. The increased action of the heated serum was again brought out, both as regards the protective and curative effects. In fact, no curative action whatever was exhibited by the unheated serum.

Having determined that the serum of rabbits injected intravenously with neosalvarsan exercised both prophylactic and curative action, the study was continued with human serum which had been used in the intraspinal treatment of patients with syphilis of the central nervous system.

A patient, weighing 57 kilograms, with tabes dorsalis, received 0.9 gm. neosalvarsan intravenously. The amount per kilogram of body weight was practically 0.015 neosalvarsan (=0.01 salvarsan). Blood was taken before, immediately after, and one hour after treatment. The serum was separated and half of each specimen was heated at 56° C. for thirty minutes. The spirochæta suspension consisted of the blood of two mice on the third day of infection, diluted to 20 c.c. 1 c.c. of each of the various dilutions of serum was mixed with 1 c.c. of the spirochæta suspension and incubated one hour at

PROTOCOL IV.
The Action of the Serum of a Patient Treated Intravenously with Neosalvarsan.

Mouse.	Serum obtained after treatment.	Dilution of serum.	Remarks.	Day after inoculation.									
				1	2	3	4	5	6	7	8	9	10
B-1	Immediately	1:1	Unheated	± W	++	++	0	0	0	0	0	0	0
B-2	Immediately	1:2	Unheated	± W	++	++	0	0	0	0	± W	± W	0
B-3	Immediately	1:4	Unheated	± W	++	Dead	—	—	—	—	—	—	—
B-4	Immediately	1:1	Heated	0	0	0	0	0	0	± W	0	0	0
B-5	Immediately	1:2	Heated	0	0	0	0	0	+	± W	0	0	0
B-6	Immediately	1:4	Heated	0	0	0	0	0	± W	±	0	0	0
C-1	1 hour	1:1	Unheated	0	0	++	± W	± W	Dead	—	—	—	—
C-2	1 hour	1:2	Unheated	± W	+	++	Dead	—	—	—	—	—	—
C-3	1 hour	1:4	Unheated	± W	++	++	0	0	0	0	0	0	± W
C-4	1 hour	1:1	Heated	0	0	0	0	0	0	0	0	0	0
C-5	1 hour	1:2	Heated	0	0	0	0	0	± W	± W	0	± W	± W
C-6	1 hour	1:4	Heated	± W	+	++	0	0	0	0	0	0	0
C-7	1 hour	1:1	Centrifugate of C-4	0	0	0	0	0	0	0	0	0	0
C-8	1 hour	1:2	Centrifugate of C-5	0	0	0	0	0	0	± W	0	0	0
C-9	1 hour	1:4	Centrifugate of C-6	±	+	++	0	0	± W	± W	+	0	0
Controls													
A-1	Before	1:1	Unheated	+	++	++	++	Dead	—	—	—	—	—
A-4	Before	1:1	Heated	+	++	++	++	0	0	0	± W	±	+
X	—	—	—	+	++	++	0	0	0	0	± W	±	0

37°. Mice were then injected intraperitoneally with 1 c.c. of the mixture. To determine whether the serum acted inside or outside the animal body, the mixtures of serum and spirochætæ, C-4, C-5, and C-6, after incubation, were centrifugalized for twenty-five minutes and all the spirochætæ thrown down, as shown by microscopical examination of the supernatant fluid. This supernatant fluid was pipetted off and the spirochætæ resuspended in normal saline and injected intraperitoneally.

The spirochæticial action of the unheated human serum obtained immediately after and one hour after treatment was practically identical. The serum obtained immediately after treatment and heated was, however, more active than the heated specimen obtained an hour later. The heating increased the action of the human serum in the same manner as it increased that of rabbit serum. The spirochætæ acted on by the serum *in vitro* and centrifugalized free from serum produced infection in mice only in the same degree as did the corresponding dilutions in which both serum and spirochætæ were injected. From this it follows that the spirochæticial action took place in the test-tube, though this action could be brought out only after the treated spirochætæ were injected into susceptible animals, since the organisms were actively motile after their treatment. Castelli³ in a similar experiment noted that dilute solutions of neosalvarsan in normal serum exerted in test-tubes a spirochæticial action upon *Spirochæta recurrentis* and *Spirochæta gallinarum*, and the effect was demonstrable only after the microörganisms were injected into animals. He concluded that the salvarsan was attached directly to the protoplasm of the parasites, and that the parasiticial action was probably completed by the body cells.

PROTOCOL V.

The Duration of the Spirochæticial Action of the Serum of a Neosalvarsan-Treated Patient.

Mouse.	Serum obtained after treatment.	Dilution of serum.	Day after inoculation			
			1	2	3	4
C—1	1 hour	1 : 1	0	0	0	0
C—2	1 hour	1 : 2	0	0	0	0
C—3	1 hour	1 : 4	0	± w	+	0

³ Castelli, G., *Ztschr. f. Chemotherap., Orig.*, 1912, i, 122.

Mouse.	Serum obtained after treatment.	Dilution of serum.	Day after inoculation.			
			1	2	3	4
D—1	3 hours	1 : 1	o	o	o	o
D—2	3 hours	1 : 2	o	o	o	o
D—3	3 hours	1 : 4	± w	±	++	o
E—1	6 hours	1 : 1	o	o	o	o
E—2	6 hours	1 : 2	o	o	+	o
E—3	6 hours	1 : 4	±	+	o	o
F—1	11 hours	1 : 1	o	± w	+	o
F—2	11 hours	1 : 2	± w	+	+	o
F—3	11 hours	1 : 4	+	+	o	Dead
G—1	24 hours	1 : 1	±	+++	++	o
G—2	24 hours	1 : 2	±	+++	+++	o
G—3	24 hours	1 : 4	+	+++	++++	o
Controls						
A	Before	1 : 1	+	+++	++	o
X	—	—	± w	+++	±	o

Some weeks later the patient used in protocol IV again received 0.9 gm. of neosalvarsan intravenously. Blood was taken before, and one, three, six, eleven, and twenty-four hours after treatment. The serum was separated and heated at 56° C. for thirty minutes. Various dilutions of each bleeding were allowed to act on the spirochætæ, and the mixture injected into mice, as in the previous experiments.

The spirochæticidal action of the heated serum decreased in proportion to the time that elapsed between treatment and bleeding, some spirochæticidal action being still present eleven hours after treatment, while after twenty-four hours the serum showed no more effect than normal serum. The Abelin⁴ diazo reaction for salvarsan was applied to the serum used in this experiment. One cubic centimeter of each specimen was tested. A deep rose red ring is the typical reaction. The results are shown in protocol V-A.

PROTOCOL V-A.

The Abelin Diazo Reaction in the Serum Used in Protocol V.

Serum.	Obtained after treatment.	Reaction.
C	1 hour	Deep red ring.
D	3 hours	Moderate red ring.
E	6 hours	Orange red ring.
F	11 hours	Light orange ring.
G	24 hours	Green brown ring.
A	Before	Green brown ring.

⁴ Abelin, J., *München. med. Wchnschr.*, 1912, lix, 81.

The color reaction decreased in proportion with the spirochæticidal action of the serum. A slight color reaction was still demonstrable in the eleven hour serum, while the twenty-four hour specimen and serum before treatment reacted alike. In another experiment in which the spirochæticidal action of the serum was slightly less marked, the diazo color reaction and the spirochæticidal action were also parallel. Hence, the experiments suggest that the action of the serum on the spirochæta is due to its salvarsan content.

PROTOCOL VI.

Comparison of Action of Serum of Patients Treated with Salvarsan and Neosalvarsan.

Mouse.	Serum obtained after treatment.	Dilution of serum.	Heated or unheated	Day after inoculation.					
				1	2	3	4	5	6
B-1	10 minutes	1:1	Heated	0	± w	±	0	0	0
B-2	10 minutes	1:2	Heated	± w	++	+++	± w	Dead	—
B-3	10 minutes	1:4	Heated	+	+++	+++	0	0	0
B-4	10 minutes	1:1	Unheated	± w	+	++	± w	± w	0
C-1	1 hour	1:1	Heated	± w	+	±	0	0	0
C-2	1 hour	1:2	Heated	±	+	+++	± w	0	0
C-3	1 hour	1:4	Heated	+	++	++++	++	± w	++
C-4	1 hour	1:1	Unheated	0	+	+	0	0	0

Neosalvarsan Serum.

BB-1	10 minutes	1:1	Heated	0	0	0	0	0	0
BB-2	10 minutes	1:2	Heated	± w	+	++	0	0	0
BB-3	10 minutes	1:4	Heated	+	++	++	0	0	0
BB-4	10 minutes	1:1	Unheated	0	0	0	0	0	0
CC-1	1 hour	1:1	Heated	0	+	++	0	0	0
CC-2	1 hour	1:2	Heated	± w	++	+	0	0	—
CC-3	1 hour	1:4	Heated	±	+++	++	0	0	—
CC-4	1 hour	1:1	Unheated	± w	++	+++	+++	± w	—
Controls									
A-1	Before	1:1	Unheated	±	++	++++	+	0	0
AA-1	Before	1:1	Unheated	+	+++	++++	++	Dead	—
X	—	—	—	+	+++	+++	Dead	—	—

A patient weighing 67 kilograms, with latent secondary syphilis, received 0.5 gm. salvarsan, and another weighing 57 kilograms, with tabes dorsalis, received 0.75 gm. neosalvarsan (corresponding to 0.5 gm. salvarsan) intravenously. The dose of salvarsan per kilogram for the first was 0.0075 gm., and for the second 0.0088 gm. Blood from each was taken before, ten minutes after, and one hour after treatment. Half of each specimen of the serum was heated at 56° C. for thirty minutes. The experiments were as before.

The results indicate that the serum of patients treated with neosalvarsan exerts slightly more spirochæticidal action on *Spirochæta duttoni* than that of patients treated with salvarsan. This difference may be due to the fact that neosalvarsan is more soluble than salvarsan. When the alkaline salvarsan is brought into contact with the blood, a part is precipitated and this precipitate is probably deposited in the organs. The neosalvarsan, being neutral, is not precipitated, and hence more of it probably remains in the blood for a short period after the intravenous injection. To test this point, two sera were submitted to the Abelin diazo reaction for salvarsan. Various dilutions, ranging between whole serum and 1 to 32 were prepared and one cubic centimeter of each dilution was tested.

PROTOCOL VI-A.

The Abelin Diazo Reaction in the Sera Used in Protocol VI.

Dilution of serum.	Salvarsan serum. Serum B.	Neosalvarsan serum. Serum BB.
1 : 1	Orange red ring	Deep red ring.
1 : 2	Light orange red ring	Orange red ring.
1 : 4	Red brown ring	Light orange red ring.
1 : 8	Faint red brown ring	Red brown ring.
1 : 16	Green brown ring	Green brown ring.
1 : 32	Green brown ring	Green brown ring.
	Serum C	Serum CC.
1 : 1	Orange red ring	Deep red ring.
1 : 2	Light orange red ring	Light orange red ring.
1 : 4	Faint red brown ring	Red brown ring.
1 : 8	Green brown ring	Green brown ring.
1 : 16	Green brown ring	Green brown ring.
1 : 32	Green brown ring	Green brown ring.
	Serum A	Water.
1 : 1	Brown ring	Green brown ring.

The results indicate that more salvarsan was present in the serum of the patient treated with neosalvarsan than in the one treated with salvarsan. The difference was more marked in the blood drawn ten minutes after treatment than one hour later. The poorer salvarsan content probably explains the smaller spirochæticidal action in the latter serum. This difference need not necessarily apply to *Treponema pallidum*, for Castelli⁵ showed that while the curative dose of salvarsan and neosalvarsan, computed in

⁵ Castelli, G., *loc. cit.*, p. 321.

weight of salvarsan, was the same for *Spirochæta recurrentis* infections in mice, the rule did not hold for the pallidum, for twice as much neosalvarsan as salvarsan was required to cure syphilis in rabbits.

PROTOCOL VII.

The Effect of Adding Normal Serum to Heated Salvarsanized Serum.

Mouse.	Serum B obtained 10 minutes after treatment.	Serum A obtained before treatment.	Normal saline.		Amount of mixture injected.	Day after inoculation.				
						1	2	3	4	5
B—1	Heated 1.0 c.c.	—	—	1 c.c. spirochaeta suspension added to each tube. Incubation at 37° C. 1 hour.	1.0 c.c.	0	0	0	0	0
B—2	0.5 c.c.	—	0.5 c.c.		1.0 c.c.	0	0	0	0	±w
B—3	0.25 c.c.	—	0.75 c.c.		1.0 c.c.	±w	Dead	—	—	—
	Un-heated									
B—4	1.0 c.c.	1.0 c.c.	—		1.5 c.c.	0	±w	±w	+	0
B—5	0.5 c.c.	0.5 c.c.	—		1.0 c.c.	±w	±w	++	±w	0
B—6	0.25 c.c.	0.25 c.c.	0.5 c.c.		1.0 c.c.	±	++	++++	++++	Dead
	Heated									
B—7	1.0 c.c.	1.0 c.c.	—		1.5 c.c.	0	0	0	0	0
B—8	0.5 c.c.	0.5 c.c.	—		1.0 c.c.	0	0	+	0	±
B—9	0.25 c.c.	0.25 c.c.	0.5 c.c.		1.0 c.c.	±w	+	++	Dead	—
	Un-heated									
B—10	1.0 c.c.	—	—		1.0 c.c.	±w	++	+++	Dead	—
B—11	0.5 c.c.	—	0.5 c.c.	1.0 c.c.	±w	++	+++	0	0	
	Un-heated									
B—12	1.0 c.c.	1.0 c.c.	—	1.5 c.c.	±w	+	++	±w	0	
B—13	0.5 c.c.	0.5 c.c.	0.5 c.c.	1.0 c.c.	±w	++	++++	+	Dead	
Controls										
A—I	—	1.0 c.c.	—	1.0 c.c.	±w	+	+++	+	0	
X	—	—	1.0 c.c.	1.0 c.c.	+	+++	++++	++++	Dead	

A patient, weighing 57 kilograms, with tabes dorsalis, received 0.75 gm. of neosalvarsan (corresponding to 0.5 gm. of salvarsan) intravenously. Blood was obtained immediately before treatment (serum A) and ten minutes after treatment (serum B). The serum was separated and one half of each specimen heated at 56° C. for one half hour. The suspension of spirochætæ was similar to that of previous experiments. Several sets of tubes were set up. In the first the spirochætæ were mixed with heated serum B, in the second with heated serum B and unheated serum A, in the third with heated serum B and heated serum A, in the fourth with unheated serum B, and in the fifth with unheated serum B and unheated serum A. After incubation for one hour, the various mixtures were injected into mice, each mouse receiving an amount which contained 0.5 c.c. of the original spirochætæ suspension.

The spirochæticidal action of the salvarsan-containing serum, increased by heating, was partly abolished by the addition of unheated

normal serum. The addition of heated normal serum to heated salvarsanized serum did not diminish appreciably the action of the salvarsanized serum. The addition of unheated normal serum to unheated salvarsanized serum did not increase its spirochæticidal action. These results indicate that the increased spirochæticidal action of heated salvarsanized serum, as compared with the same serum unheated, is due, in part at least, to the destruction of some inhibiting substance which is contained in both normal and salvarsanized serum. It is probable that the increased activity of heated serum is not due entirely to the destruction of this inhibiting substance, since, if this were the case, the addition of an equal amount of unheated normal serum should supply sufficient inhibiting substance to neutralize the effect produced by heating; the mixture would, therefore, behave exactly like the unheated salvarsanized serum. This, however, was not the case, for the mixture of heated salvarsanized serum and unheated normal serum was always more spirochæticidal than the unheated salvarsanized serum alone. Besides, protocol III, in which both the prophylactic and curative effects of the salvarsanized rabbit serum were exerted within the bodies of mice, indicates that heating increases the action of the serum. If the increased activity were entirely due to the destruction of some inhibiting substance by heating, it would seem that the mouse serum should supply the inhibiting substance, and both unheated and heated serum act alike. The heated serum, however, always excited a more marked action than the unheated serum. It is probable, therefore, that the effect of heating, besides destroying some inhibiting substance in normal serum, increases the spirochæticidal action of the salvarsan-serum mixture by producing some change in the mixture.

PROTOCOL VIII.
The Effect of Adding Cerebrospinal Fluid to Heated Salvarsanized Serum.

Mouse.	Serum C obtained one hour after treatment.	Cerebro- spinal fluid S.	Serum A obtained before treatment.	Normal saline.	Incubation at 37° C. 1 hour.	Day after inoculation.							
						1	2	3	4	5	6	7	8
C-1	Heated 1.0 c.c.	—	—	—	1.0 c.c.	0	0	0	0	0	0	0	± W
C-2	0.5 c.c.	—	—	0.5 c.c.	1.0 c.c.	0	0	0	0	0	0	0	0
C-3	0.25 c.c.	—	—	0.75 c.c.	1.0 c.c.	± W	+	++	± W	± W	0	0	0
C-4	1.0 c.c.	Unheated	—	—	1.5 c.c.	0	0	0	0	0	0	± W	±
C-5	0.5 c.c.	1.0 c.c.	—	—	1.0 c.c.	0	0	0	0	0	± W	+	± W
C-6	0.25 c.c.	0.5 c.c.	—	0.5 c.c.	1.0 c.c.	± W	+	± W	0	0	0	0	± W
C-7	1.0 c.c.	Heated	—	—	1.5 c.c.	0	0	0	0	0	0	0	± W
C-8	0.5 c.c.	1.0 c.c.	—	—	1.0 c.c.	0	0	0	0	± W	+	0	0
C-9	0.25 c.c.	0.5 c.c.	—	0.5 c.c.	1.0 c.c.	± W	± W	++	0	0	0	± W	±
C-10	1.0 c.c.	Unheated	Unheated	—	1.5 c.c.	0	0	0	± W	Dead	—	—	—
C-11	0.5 c.c.	—	0.5 c.c.	—	1.0 c.c.	± W	± W	+	0	0	—	± W	0
C-12	0.25 c.c.	—	0.25 c.c.	0.5 c.c.	1.0 c.c.	± W	+	+	0	0	0	± W	± W
C-13	1.0 c.c.	—	Heated	—	1.5 c.c.	0	0	0	0	0	± W	+	± W
C-14	0.5 c.c.	—	1.0 c.c.	—	1.0 c.c.	0	0	0	0	0	0	± W	± W
C-15	0.25 c.c.	—	0.5 c.c.	0.5 c.c.	1.0 c.c.	± W	+	0	0	0	0	0	0
C-16	1.0 c.c.	Unheated	—	—	1.0 c.c.	0	0	0	0	±	++	++	+
C-17	0.5 c.c.	—	—	0.5 c.c.	1.0 c.c.	± W	±	++	+	0	0	0	± W
C-18	0.25 c.c.	—	—	0.75 c.c.	1.0 c.c.	±	++	+	0	0	0	0	0
Controls													
S-1	—	Unheated	—	—	1.0 c.c.	±	+	++	0	0	0	± W	0
S-2	—	1.0 c.c.	—	—	1.0 c.c.	±	+	+	0	0	0	0	0
A	—	Heated	—	—	1.0 c.c.	±	+	++	++	0	0	0	0
X	—	1.0 c.c.	—	1.0 c.c.	1.0 c.c.	+	+	++	++	0	0	0	0

A patient, weighing 56 kilograms, with *tabes dorsalis*, received 0.5 gm. of salvarsan intravenously. Blood was obtained before treatment (serum A) and one hour after (serum C). Twenty-four hours later cerebrospinal fluid was obtained from the same patient. This fluid contained an excess of globulin. Half of each specimen of the serum and half of the cerebrospinal fluid were heated at 56° C. for thirty minutes. An experiment was made in the same manner as the preceding one, with the addition of two sets of tubes, viz., heated salvarsanized serum with unheated cerebrospinal fluid, and heated salvarsanized serum with heated cerebrospinal fluid.

The object was to determine whether cerebrospinal fluid contained an inhibiting substance similar to that in unheated serum. It was necessary to duplicate protocol VII as a control. The addition of normal unheated serum decreased somewhat the spirochæticidal action of the heated salvarsanized serum, as in the preceding experiment. The addition of heated normal serum and of cerebrospinal fluid, both heated and unheated, to the heated salvarsanized serum had only slight effect, shown by delayed infections in the mice receiving mixtures of 0.5 of a cubic centimeter. All the mice (except C—2) which failed to show early infection, developed slight, short infections between the fifth and eighth days. We have frequently noticed that the late developing infections are usually slight and irregular in time of development, so that little weight can be placed upon them beyond showing that all spirochætæ were not killed. The apparent spirochæticidal action of the cerebrospinal fluid alone is probably due to the fact that very little serum was present in the mixture during the period of incubation. We have repeatedly noticed that spirochætæ incubated for one hour in normal salt solution failed to produce as severe an infection as organisms after incubation in normal serum. Under the dark-field microscope, at the end of one hour's incubation, many of the spirochætæ incubated with salt solution appeared granular and had lost their motility, while those incubated with normal serum were actively motile. As cerebrospinal fluid is hypotonic, it is probable that many of the spirochætæ incubated in it were permanently injured. When serum was present in the mixture, the hypotonicity was diminished; hence the spirochætæ were injured less. The inhibitory substance present in normal serum and destroyed by heating at 56° C. is absent from cerebrospinal fluid, even when the fluid contains an abnormal amount of globulin.

In so far as the results obtained with one variety of spirochæta can be applied to another variety, the work here presented suggests that the serum of patients treated with salvarsan should have a distinct spirochæticidal action on *Treponema pallidum*. Probably the best way to treat many of the manifestations of syphilitic infection of the central nervous system is to introduce the therapeutic agent directly into the cerebrospinal fluid, which carries it directly to the diseased tissue. We⁶ have been able to demonstrate that the subdural injection of the serum of salvarsan-treated patients has a definite curative action in syphilitic disease of the central nervous system. The above experiments with *Spirochæta duttoni* suggest that this curative action is due to the direct spirochæticidal action of the salvarsanized serum.

SUMMARY.

1. The serum of rabbits treated intravenously with neosalvarsan, and of syphilitic patients treated intravenously with salvarsan or neosalvarsan, has a definite spirochæticidal action upon *Spirochæta duttoni*.
2. Although this spirochæticidal action is exerted *in vitro*, it can be demonstrated only after the treated spirochæte are injected into susceptible animals.
3. A curative action of the serum of neosalvarsan-treated rabbits is exercised on mice infected with *Spirochæta duttoni*.
4. The spirochæticidal action of the serum of salvarsan-treated rabbits and patients is markedly increased by heating at 56° C. for thirty minutes.
5. The increased spirochæticidal action produced by heating is due in part to the destruction of some inhibitory substance contained in normal serum and in part to a direct effect of the heat upon the serum and salvarsan mixture.
6. Cerebrospinal fluid does not contain the inhibitory substance present in normal unheated serum.

⁶ Swift, H. F., and Ellis, A. W. M., *loc. cit.*

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